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Some Biological, Chemical and Physical Properties of Sulfuryl Fluoride[1] as an Insecticidal Fumigant[2]

E. E. KENAGA, *The Dow Chemical Co., Midland, Michigan*

The ideal requirements of a good fumigant for the control of structural and commodity insect pests are rather exacting. The fumigant must be toxic to insects under all temperature and exposure conditions, non-explosive, easily dispersed, essentially non-reactive, relatively non-sorptive in commodities and able to penetrate rapidly through infested materials. Sulfuryl fluoride is a fumigant found in this laboratory to possess the above properties and to be of outstanding value for the control of insects. Stewart (1956) has reported on the use of sulfuryl fluoride for the control of the drywood termite, *Kalotermes minor* Hagen.

PHYSICAL AND CHEMICAL PROPERTIES OF SULFURYL FLUORIDE.—Sulfuryl fluoride (SO_2F_2) is a non-flammable, colorless, odorless compound which boils at −55.2° C. at 760 mm. pressure. This material has a boiling point lower than any commercial insecticidal fumigant. The boiling point and vapor pressure of sulfuryl fluoride compared with some common fumigants at various temperatures are given in table 1. At ordinary room temperature (25° C.), sulfuryl fluoride exerts a pressure of 260 pounds per square inch or about 18 atmospheres. At −5° C., the vapor pressure of SO_2F_2 is nearly three times that of methyl bromide at 40° C. A high vapor pressure is particularly useful for promoting the attainment of gas-air equilibrium, Sulfuryl fluoride is easily dispersed even under low temperature conditions. A high vapor pressure also aids gaseous penetration of commodities and structural materials The vapor pressure and molecular weight govern the amount of any fumigant required to saturate air at a given temperature. Air saturation data for sulfuryl fluoride and some common fumigants at various temperatures are presented in table 2. Under practical fumigation conditions, extremely high concentrations are possible without saturating the air with sulfuryl fluoride or methyl bromide. Other fumigants shown in this table saturate air at much lower concentrations, sometimes in quantities insufficient to control insects. Sulfuryl fluoride is heavier than air as a gas and heavier than water as a liquid. Comparative data on the specific gravity and the latent heat of vaporization of various fumigants are given in table 3. This latter property governs the ease of volatilization. The solubility of sulfuryl fluoride in water and in several other solvents at various temperatures was determined by the gas volume loss in a standard Orsat apparatus by bubbling gaseous sulfuryl fluoride through the solvents at atmospheric pressure. These solubility data appear in table 4. Sulfuryl fluoride had a very low solubility in most materials used in this study. However, sulfuryl fluoride is soluble in methyl bromide in all proportions at −78° C.

Sulfuryl fluoride may be safely used on a wide variety of household materials. The following items were noted to be undamaged by sulfuryl fluoride in these tests as judged by the lack of odor and color changes, and the lack of corrosion after a 16-hour exposure in an atmosphere containing 3 pounds of sulfuryl fluoride per 1,000 cubic feet at 80° F.:

stainless steel
brass
copper
aluminum
zinc
silver
butyl rubber
buna rubber
buna H rubber
Polyacrylon rubber
neoprene rubber
Thiokol rubber
saran rubber
natural rubber
sponge rubber
cork rubber sole
Neolite sole
Orlon thread
nylon thread
Dacron thread
acetate rayon thread
wool thread
cotton thread
many colors of wool suiting
many colors of wallpaper
six kinds of cowhide
blue suede
black and white kid leather
horsehide

INSECTICIDAL ACTIVITY OF SULFURYL FLUORIDE.—The following insects and life stages were used in these studies:

Confused flour beetle (*Tribolium confusum* Duv.)
- Adults—4 to 5 weeks old
- Eggs—2 days old

Granary weevil (*Sitophilus granarius* (L.))
- Adults—1 to 2 weeks old
- Pupae—in wheat infested 30 days previously
- Larvae—in wheat infested 16 days previously
- Eggs—1 to 14 days old

Lesser grain borer (*Rhyzopertha dominica* (F.))
- Adults—1 to 2 weeks old
- Pupae—in wheat infested 30 to 32 days previously
- Eggs—6 days old

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[1] Sulfuryl fluoride is known as Vikane, a trademark of the Dow Chemical Company, Midland, Michigan.

[2] Accepted for publication Dec. 13, 1956.

Table 1.—Boiling point and vapor pressures of SO_2F_2 and some common fumigants.

Fumigant	B.P. °C. 760 Mm.	Vapor Pressure[a]—Mm. −5° C.	10° C.	25° C.	40° C.
SO_2F_2	−55.2	6,255.0	9,150.0	13,442.0	19,646
CH_3Br	4.5	550.0	940.0	1,580.0	2,500
CS_2	46.2	98.0	195.0	361.6	620
CCl_4	76.5	24.0	54.0	114.5	210
EDC[b]	83.0	17.5	41.0	79.9	164
EDB[c]	131.6	2.7	6.7	11.6	30

[a] Calculated from "Vapor Pressures of Pure Substances" (D. R. Stull, 1947).
[b] Ethylene dichloride.
[c] Ethylene dibromide.

Saw-toothed grain beetle (*Oryzaephilus surinamensis* (L.))
Adults—various ages
Black carpet beetle (*Attagenus piceus* (Oliv.))
Larvae—3 to 5 months old of uniform size
Eggs—2 days old
Cigarette beetle (*Lasioderma serricorne* (F.))
Adults—30 days old
Mexican bean beetle (*Epilachna varivestes* Muls.)
Eggs—1 day old
Corn beetle (*Cynaeus angustus* Lec.)
Adults—various ages
Larvae—various ages
American cockroach (*Periplaneta americana* (L.))
Adults—5 months from egg stage
Eggs—1 to 14 days old
German cockroach (*Blattella germanica* (L.))
Adults—all females of various ages
Angoumois grain moth (*Sitotroga cerealella* (Oliv.))
Adults—2 to 3 days old
Larvae—in wheat infested 16 days previously
Eggs—2 days old
Mediterranean flour moth (*Anagasta kühniella* (Zell.))
Adults—30 to 35 days from egg stage
Larvae—17 days from egg stage
Southern armyworm (*Prodenia eridania* (Cram.))
Eggs —1 day old
House fly (*Musca domestica* L.)
Adults—3 days old
Pupae—3 days old

All insects were reared at 80° F. and at about 60% relative humidity. The fumigation tests were carried out in 25.5-liter vaults as described by Kenaga (1953).

At the end of the exposure to the fumigant the insects were placed in the rearing room and held for varying lengths of time. The mortality counts on the egg stage and other life stages in the grain kernels were made by comparison with the number of insects emerging from the untreated controls. Southern armyworm and Mexican bean beetle eggs were held until the untreated controls hatched in closed petri dishes in which the humidity was held constant with a saturated saline solution. Mortality counts on the adult flies were taken immediately after exposure; adult moths and German cockroaches 3 days after; adult corn beetles, saw-toothed grain beetles and American cockroaches 7 days after; and all other life stages of insects 14 days after exposure. Cold temperature tests (40° F. and 60° F.) were conducted by placing the insects in the 25.5-liter fumigation vault which was, in turn, placed in a temperature controlled 32-cubic-foot vault and held for about 6 hours for temperature conditioning of the insects before the application of the fumigant. After exposure, the insects were returned to the 80° F. rearing room and held until completion of the mortality counts. The relationship of dosage and mortality for each species was calculated on the basis of 33 individuals per test except with the cockroaches where 10 per test were used. Where the insect life stages are found within grain kernels, the emergence of the life stage was compared with the numbers emerged

Table 2.—Air saturation concentrations of SO_2F_2 and some common fumigants.

Fumigant	Air Saturated (1 Atmos.) Lbs./1,000 Cu. Ft. −5° C.	10° C.	25° C.	40° C.	P. P. M. at 1 Lb./1,000 Cu. Ft.
SO_2F_2	—[a]	—[a]	—[a]	—[a]	3,832
CH_3Br	195.5	—[a]	—[a]	—[a]	4,116
CS_2	27.9	53.9	94.9	155.3	5,147
CCl_4	13.8	29.5	59.7	103.6	2,543
EDC[b]	6.5	14.4	26.6	52.0	3,913
EDB[c]	1.9	4.4	7.3	18.1	2,071

[a] Complete displacement of all air without condensation (>200 pounds).
[b] Ethylene dichloride.
[c] Ethylene dibromide.

Table 3.—Specific gravity and latent heat of vaporization of SO_2F_2 and some common grain fumigants.

Fumigant	Spec. Gravity of Fumigant at 25° C. Compared with H_2O at 4° C.	Gas Compared with Air[a]	Latent Heat of Vaporization at B.P.—Cals. per Mole
SO_2F_2	1.342	3.52	4,600 (±5%)
CH_3Br	1.732 (0/0° C.)	3.28	5,750
CS_2	1.256	2.62	6,399
CCl_4	1.584	5.31	7,140
EDC[b]	1.246	3.41	7.652
EDB[c]	2.17	6.48	7,688

[a] Based on molecular weight of 29 for air.
[b] Ethylene dichloride.
[c] Ethylene dibromide.

Table 4.—Solubility of sulfuryl fluoride in various solvents.

Temperature—° C.	Gm. SO_2F_2/100 Gm. Material	
0	0.16	water
10	.10	water
25	.075	water
40	.07	water
0	.94	Wesson oil (cottonseed)
10	.87	Wesson oil (cottonseed)
20	.78	Wesson oil (cottonseed)
40	.59	Wesson oil (cottonseed)
25	.2	propylene glycol
25	.62	peanut oil
22	1.74	acetone
22	2.12	chloroform
22	.5	ethylene dibromide
22	.77	Stoddard's solvent
22	1.4	isopropyl formate
22	.62	propylene oxide
−78	infinity	methyl bromide

Table 5.—Toxicity of sulfuryl fluoride to various insect species and life stages (80° F., 16-hour exposure).

Insect Species	Life Stage	Ounces per 1,000 Cubic Feet			
		LD_{50}(±95% C.L.[a])		LD_{95}(±95% C.L.[a])	
Confused flour beetle	adult	3.14	(.45)	3.45	(.46)
	egg	42.7	(93.4)	70.3	(93.8)
Granary weevil	adult	0.63	(.25)	0.91	(.23)
	pupa	0.76	(.46)	0.86	(.46)
	larva	0.36	(.33)	0.86	(.33)
	egg	24.9	(45)	49.6	(44)
Lesser grain borer	adult	0.19	(.72)	0.65	(.65)
	pupa	0.60	(.47)	1.31	(.47)
	egg	8.45	(6.38)	13.70	(6.16)
Saw-toothed grain beetle	adult	0.78	(.31)	0.87	(.31)
Black carpet beetle	larva	2.08	(.63)	2.39	(.63)
	egg	42.3	(95.3)	75.8	(95.1)
Cigarette beetle	adult	0.71	(.21)	0.94	(.24)
Mexican bean beetle	egg	17.96	(5.49)	20.17	(5.72)
Corn beetle	adult	1.89	(.43)	2.31	(.44)
	larva	2.17	(1.08)	2.22	(1.07)
American cockroach	adult	.46	(.27)	.59	(.27)
	egg	19.41	(9.86)	25.80	(9.93)
German cockroach	adult	.77	(1)	1.16	(1)
Angoumois grain moth	adult	.74	(.45)	1.32	(.47)
	larva	.82	(.39)	1.50	(.40)
	egg	4.81	(2.85)	5.44	(3.0)
Mediterranean flour moth	adult	1.35	(.89)	2.14	(.86)
	larva	1.1	(.21)	2.6	(2.12)
Southern armyworm	egg	18.21	(6.74)	22.7	(6.89)
House fly	adult	.54	(.33)	.96	(.33)
	pupa	.96	(.41)	1.36	(.42)

[a] = ±95% confidence limits.

in an equivalent volume of the untreated grain. The regression line of per cent kill on dosage for each life stage of each insect species was calculated from the results of 10 to 32 separate tests. From this regression line and the mean, the LD_{50} and LD_{95} values were calculated by use of Student's "t" table. Dosages are given in terms of ounces per 1,000 cubic feet (cu. ft.) which is, coincidentally, equivalent to milligrams per liter.

The results of the space fumigation tests using sulfuryl fluoride on the various life stages representing 14 different insect species at 80° F. and for an exposure period of 16 hours are shown in table 5. Sulfuryl fluoride killed the larval, pupal and adult stages of the various insects within a two- to three-fold dosage range of each other at the LD_{95} level. In some instances the egg stage required as much as 50 times the dosage needed to kill other life stages of the same species. Eggs of the black carpet beetle, the confused flour beetle and the granary weevil were the least susceptible to sulfuryl fluoride of the species tested. The variation in egg kill by sulfuryl fluoride as shown by the 95% confidence limits was quite large. This large variation appeared to be due mainly to dosage response differences between tests conducted on separate days rather than those conducted on the same day. Further studies are needed to explain the reason for these variations in egg kill.

The egg stage of the insect is also the life form most tolerant to several commercial fumigants such as carbon tetrachloride, carbon disulfide, ethylene dichloride and benzene (Kenaga 1953). In the use of methyl bromide and ethylene oxide, the most resistant life stage was the egg stage. The LD_{95} of sulfuryl fluoride on life stages of insect species tested other than the egg stage were all less than 4 ounces per 1,000 cu. ft., which places it in the same range of insect toxicity as methyl bromide. Stewart (1956) has reported on the use of sulfuryl fluoride for the control of the drywood termite, *Kalotermes minor* Hagen, and discussed mammalian toxicity and other problems associated with structural fumigation.

There is a need by investigators in the field of space and commodity fumigation for information on the effects of temperatures and exposure periods on the dosage of all of the common fumigants required for insect kill. The information must be sufficient to make interpolations to suit the particular needs of a given fumigation. As a partial solution to this need, the LD_{50} and LD_{95} dosages for sulfuryl fluoride on the confused flour beetle adult and the black carpet beetle larva at 80, 60 and 40° F. using a 16-hour exposure and also using 16-, 5-, 2-, 1- and 0.5-hour exposures at 80° F. were determined (see table 6). Comparative information on the same exposures and temperatures using methyl bromide as the fumigant is shown in table 7. In these tests, sulfuryl fluoride was more toxic than methyl bromide against the confused flour beetle and black carpet beetle at all exposure periods and temperature conditions. Sulfuryl fluoride was more toxic to the black carpet beetle than to the confused flour beetle at 80° F. and was less toxic at 40° F. Sulfuryl fluoride was more toxic in the 16-hour exposure to the black carpet beetle than to the confused flour beetle, but

Table 6.—The effect of various temperatures and exposure periods on the insect toxicity of sulfuryl fluoride.

(Hours) Exposure	Temperature ° F.	Ounces per 1,000 Cubic Feet							
		LD_{50}(±95% C.L.[a])		LD_{95}(±95% C.L.[a])		LD_{50}(±95% C.L.[a])		LD_{95}(±95% C.L.[a])	
		Confused Flour Beetle				Black Carpet Beetle			
16	80	3.14	(.45)	3.45	(.46)	2.08	(.63)	2.39	(.63)
16	60	3.78	(.19)	4.00	(.19)	2.51	(.58)	3.36	(.56)
16	40	6.29	(.75)	7.38	(1.76)	9.68	(3.84)	13.00	(3.84)
16	80	3.14	(.45)	3.45	(.46)	2.08	(.63)	2.39	(.63)
5	80	6.86	(1.36)	8.06	(1.47)	5.19	(3.04)	6.75	(3.04)
2	80	12.77	(2.88)	14.32	(4.32)	11.71	(2.24)	14.45	(2.24)
1	80	19.36	(2.24)	22.56	(2.40)	18.40	(13.12)	26.32	(13.12)
0.5	80	36.16	(15.84)	43.84	(15.84)	27.84	(11.20)	42.08	(10.72)

[a] = ±95% confidence limits.

Table 7.—The effect of various temperatures and exposure periods on the insect toxicity of methyl bromide.

(Hours) Exposure	Temperature ° F.	Ounces per 1,000 Cubic Feet							
		LD^{50}(%95% C.L.[a])		LD^{95}(±95% C.L.[a])		LD^{50}(±95% C.L.[a])		LD^{95}(±95% C.L.[a])	
		Confused Flour Beetle				Black Carpet Beetle			
16	80	3.29	(.44)	3.71	(.44)	3.60	(.30)	4.02	(.48)
16	60	3.87	(.48)	4.74	(.48)	4.21	(1.44)	4.70	(1.76)
16	40	9.22	(3.04)	11.95	(3.20)	11.10	(2.88)	14.54	(3.04)
16	80	3.29	(.44)	3.71	(.44)	3.60	(.30)	4.02	(.48)
5	80	8.34	(1.22)	9.49	(1.71)	8.64	(3.20)	9.60	(3.36)
2	80	20.03	(8.80)	22.75	(9.12)	18.72	(1.20)	24.48	(1.25)
1	80	29.92	(11.52)	36.00	(14.4)	29.92	(4.80)	36.16	(5.76)
0.5	80	46.08	(14.08)	55.20	(14.24)	48.58	(23.36)	53.90	(23.36)

[a] = ±95% confidence limits.

of about the same toxicity in short exposure tests. With the use of both methyl bromide and sulfuryl fluoride, considerably more fumigant was necessary for the same degree of insect kill with a decrease from 60 to 40° F. than was necessary for a decrease from 80 to 60° F.

Penetration of the fumigant into various commodities in toxic amounts is important for the control of insects in stored products. For the purpose of ascertaining the penetration properties of the fumigants, a 10-inch length of 1-inch diameter pipe with one end sealed was filled with various commodities. Slotted plastic vials containing insects were placed at the 9-inch, 5-inch and 1-inch depths in the commodity (Kenaga 1953). All commodities were then packed in the pipe by applying uniform pressure and by shaking. The pipe was placed upright and the fumigant was applied in the same manner as in the space fumigation tests. The commodities chosen for the penetration studies were selected on a basis of pH and particle size as well as a variety of chemical and sorptive properties.

Methyl bromide is well known in commercial practice as a fumigant possessing excellent penetrating properties. It is able to reach and kill insects in all types of commodities at reasonable and relatively low toxicant concentrations. However, for the purposes of comparison, sulfuryl fluoride and methyl bromide were studied at dosages which would clearly indicate small or marginal differences between the two fumigants. A dosage of 8 ounces per 1,000 cubic feet at 80° F. for a 16-hour exposure period was employed to compare these fumigants. Black carpet beetle larvae and confused flour beetle adults were used as indicators of lethal penetrating dosages in the various commodities. The results of these tests are shown in table 8. Sulfuryl fluoride killed both insect species at all depths in all commodities tested. Methyl bromide did likewise in the powdered milk, but in none of the other commodities tested. A comprehensive study of the reactions of methyl bromide with the proteins, water, starch and fat of wheat have been discussed by Winteringham (1955) and Bridges (1955). There was no evidence to indicate that these reaction products are toxic to mammals; however, it may account for some of the fumigant lost in the penetration of wheat and proteinaceous materials. Possible residues resulting from the similar use of sulfuryl fluoride have not been fully determined as yet. Organic materials such as hardwood flour and ground tobacco also sorbed enough methyl bromide to reduce the kill of insects at the 5- and 9-inch depths. Thus, at the dosage used, sulfuryl fluoride was superior to methyl bromide in the penetration of a variety of commodities.

The same insect species and test methods were used to test the penetrating ability of methyl bromide and sulfuryl fluoride in three types of soil. Results from these tests are shown in table 9. Sand, clay and peat were selected as representatives of widely varying types of soil. Methyl bromide appeared to be sorbed the most by the organic peat and also was sorbed more in dry than in wet soils. Based on insect kill, sulfuryl fluoride penetrated dry better than wet soils. It penetrated organic about as well as inorganic soils. Sulfuryl fluoride was superior to methyl bromide in penetration in nearly all of the soil tests and was considerably more effective in penetrating organic soils.

Tests with Sulfuryl Fluoride on Organisms

Table 8.—The relative penetration properties of sulfuryl fluoride and methyl bromide in various commodities at 8 ounces per 1,000 cubic feet (80° F.—16-hour exposure).

Commodity	Average Particle Size (Microns)	pH Water (Slurry)	Depth Insects Buried (Inches)	Per Cent Mortality			
				CH_3Br		SO_2F_2	
				BCB[a]	CFB[b]	BCB	CFB
"Mike" sulfur	5	7.6	1	100	100	100	100
			5	98	100	100	100
			9	55	100	100	100
Wheat gluten	30	5.5	1	100	100	100	100
			5	3	3	100	100
			9	3	3	100	100
Hardwood flour	10	4.9	1	82	100	100	100
			5	0	15	100	100
			9	0	6	100	100
Powdered milk	10	6.7	1	100	100	100	100
			5	100	100	100	100
			9	100	100	100	100
Barden clay	8	5.3	1	100	100	100	100
			5	100	100	100	100
			9	61	100	100	100
White wheat flour	25	5.4	1	100	100	100	100
			5	12	85	100	100
			9	3	9	100	100
Ground tobacco	5	6.5	1	100	100	100	100
			5	100	61	100	100
			9	27	0	100	100

[a] Black carpet beetle larva.
[b] Confused flour beetle adult.

Table 9.—**The relative penetration properties of sulfuryl fluoride and methyl bromide in various types and moistures of soils (80° F.—16-hour exposure).**

Type of Soil	Per Cent H_2O by Weight	Depth Insects Buried in Soil (Inches)	Ounces Fumigant per 1,000 Cu. Ft. Applied for Approximate 100% Mortality			
			SO_2F_2		CH_2Br	
			BCB[a]	CFB[b]	BCB	CFB
sand	0	1	*4*[c]	*4*	4	4
		5	*4*	*4*	4	6
		9	*4*	*4*	4	6
clay loam	0.5	1	*4*	4	6	*4*
		5	*4*	6	8	8
		9	*4*	6	8	8
clay loam	7.8	1	*4*	4	*4*	*4*
		5	*4*	6	6	*4*
		9	*4*	6	6	*4*
clay loam	15.7	1	*4*	*4*	*4*	*4*
		5	*4*	6	6	4
		9	4	6	6	6
peat	2.0	1	*4*	*4*	6	6
		5	*4*	*4*	8	8
		9	*4*	*4*	16	16
peat	47.5	1	*6*	*6*	*6*	*6*
		5	*6*	*6*	*6*	*6*
		9	*6*	*6*	*6*	*6*

[a] Black carpet beetle larva.
[b] Confused flour beetle adult.
[c] Figures in italics indicate lowest concentration tested.

OTHER THAN INSECTS.—All organisms in these tests were fumigated in the same type of 25.5-liter fumigation vaults used in the insecticidal tests employing the same application techniques.

Aquatic Organisms.—Goldfish (*Carassius auratus* (L.)), lake emerald shiner (*Notropis a. atherinoides* Rafinesque), and undetermined species of ram's horn snails, turtles and frog tadpoles were killed when placed in 4 liters of water and exposed to a dosage of 24 ounces per 1,000 cu. ft. applied in the fumigation vault air space for a 16-hour exposure at 80° F.

Germination of Various Weed, Grain and Crop Seeds.—These tests were conducted by placing 50 seeds of each species or variety tested in the fumigation vault, exposing them for 16 hours at 80° F. Each species of seed was tested at dosages of 80, 40 and 16 ounces per 1,000 cu. ft. The exposed seeds were then aired for 1 to 2 hours, placed on wet filter paper and covered to keep the moisture in for 3 days, at which time germination counts were made and compared with germination of the untreated seed. The following plant seeds were fumigated:

Rough rice (*Oryza sativa* L.) (13.6% moisture)
White soft and red hard wheat (*Triticum aestivum* L.) (12.6% moisture)
Oats (*Avena sativa* L.) (9.3% moisture)
Barley (*Hordeum vulgare* L.) (15.7% moisture)
Rye (*Secale cereale* L.)
Corn (*Zea mays* L.) (12.3% moisture)
Milo sorghum and sorghum (*Sorghum vulgare* Pers.) (12.5–14.5% moisture)
Flax (*Linum usitatissimum* L.)
Proso millet (*Panicum miliaceum* L.)
Black valentine, navy and golden wax beans (*Phaseolus vulgaris* L.) (16–18% moisture)
Lima bean (*Phaseolus limensis* MacFad.)
Horse bean (*Vicia Faba* L.)
Black-eye cowpea (*Vigna sinensis* Endl. (L.))
Red and sugar beets (*Beta vulgaris* L.)
Rape (*Brassica Rapa* L.)
Onion (*Allium Cepa* L.)
Cucumber (*Cucurbita sativus* L.)
Radish (*Raphanus sativus* L.)
Grimm alfalfa (*Medicago sativa* L.)
Leafy spurge (*Euphorbia Esula* L.)
Common milkweed (*Asclepias syriaca* L.)
Narrow-leaf sorrel (*Rumex Acetosella* L.
Red root pigweed (*Amaranthus retroflexus* L.)
Sweet clover (*Melilotus* L. sp.)
Red clover (*Trifolium pratense* L.)
Ragweed (*Ambrosia artemisiifolia* L.)
Johnson grass (*Sorghum halepense* (L.) Pers.)
Crabgrass (*Digitaria sanguinalis* (L.) Scop.)
Brome grass (*Bromus inermis* Leyss.)
Quackgrass (*Agropyron repens* L.)

All of the treated seeds germinated as well as the untreated checks. The moisture content of the seeds are given where known; however, the dosage, seed moisture and seed germination relationships need further study.

Fresh Fruits, Vegetables, Tubers and Growing Plants.—The plant materials used in these tests were exposed for 2 hours at 80° F. and at dosages of 16 and 48 ounces per 1,000 cu. ft. The results of the tests are shown in table 10. The effects on the plants were those in addition to the natural aging process occurring on the untreated produce. The effect of sulfuryl fluoride was quite severe on fresh fruits and vegetables in contrast to practically no effect on dormant seeds as shown in the previous tests. The major effects seemed to be a chemical burning on the outer surface of the plant or plant produce at the higher concentrations and a maturing effect which resulted in quickened rotting or desiccation at both dosages.

The Comparative Sorption of Sulfuryl Fluoride and Methyl Bromide in Wheat.—For the purpose of comparison, the conditions under which these tests were conducted were intentionally exaggerated, the temperature being low (48° F.), the exposure long (14 days) and the dosage fairly high (96, 64 and 32 ounces per 1,000 cu. ft.). In these tests, 30 pounds of wheat of a 13 to 14.5% moisture content were placed in a 25.5-liter vault, making about a 50% air space load. The fumigant was then introduced into the vault as a vapor under vacuum. The vault was brought immediately to atmospheric pressure and rolled in such a manner as to insure a quick distribu-

Table 10.—**The effect of sulfuryl fluoride on fruits, vegetables, tubers and plants (80° F.—2-hour exposure).**

Ounces 1,000 Cu. Ft.	Plant Tested		Effect on Plant or Plant Produce
	Species	Stage	
48	Apple	Fruit	Browns skin and flesh, fruit decayed more rapidly
16	Apple	Fruit	Browns skin and flesh, fruit ½ rotten 10 days
48	Orange	Fruit	Browns skin, desiccated, more rapid decay
16	Orange	Fruit	Color O.K., desiccated 10% of skin
48	Potato	Tuber	Slight russeting effect
16	Potato	Tuber	Very slight russeting effect
48	Celery	Stalk	Spotted, brown, desiccated rapidly
16	Celery	Stalk	Spotted, brown, desiccated rapidly
48	Tomato	Fruit	Skin spotted, brown, fruit spoiled quickly
16	Tomato	Fruit	Skin not spotted, fruit spoiled quickly
48	Tomato	Plant	Dead a few days after exposure
16	Tomato	Plant	Dead a few days after exposure
48	Corn	Plant	Killed all but roots in a few days
16	Corn	Plant	Killed all but roots in a few days

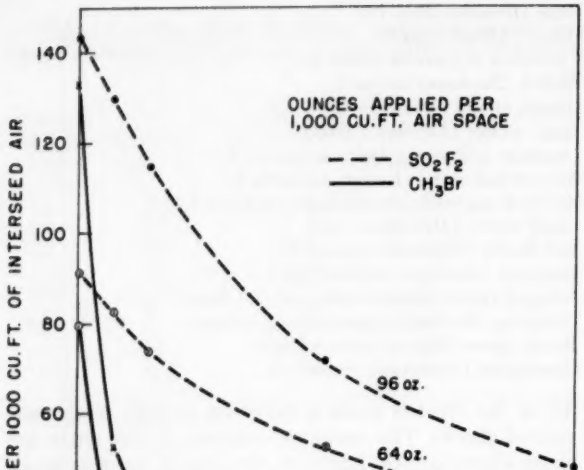

FIG. 1—The relative sorption of sulfuryl fluoride and methyl bromide in wheat at 40° F.

tion of the gas throughout the grain. The three concentrations of each type of fumigant were determined by means of a thermoconductivity unit[3] at daily intervals after application as shown by Kenaga (1955). Depending upon the concentration and length of exposure, sulfuryl fluoride was sorbed to the extent of 14 to 43% of the amount of methyl bromide sorbed under similar circumstances (see figure 1).

SUMMARY AND CONCLUSIONS.—Sulfuryl fluoride is a nonflammable, colorless, odorless compound which boils at −55.2° C. at 760 mm. It has a high vapor pressure and a low heat of vaporization. These properties are of value in the rapid distribution and penetration of the gas and in the ease of application. Sulfuryl fluoride is stable in air, showing no observable reaction with metals, rubbers, plastics, paper, leather, cloths, wallpaper and many other household items based on odor, color or corrosive changes in the fumigated commodities.

Sulfuryl fluoride was tested as a fumigant against the various life stages of insects representing 14 species in which the LD^{95} varied from 0.65 ounces per 1,000 cu. ft. against the lesser grain borer adult to 75.8 ounces per 1,000 cu. ft. against the egg of the black carpet beetle. A condensed tabulation of these results, based on the LD^{95} dosage for the various life stages, follows:

Life Stage	No. of Species Tested	Ounces per 1,000 Cubic Feet for LD^{95}	
		Most Resistant Species	Least Resistant Species
adult	11	3.45 (confused flour beetle)	0.65 (lesser grain borer)
pupa	3	1.36 (house fly)	0.86 (granary weevil)
larva	5	2.60 (Mediterranean flour moth)	0.86 (granary weevil)
egg	8	75.80 (black carpet beetle)	5.44 (Angoumois grain moth)

Sulfuryl fluoride kills all insect life stages. However, the adult, pupal and larval stages are more susceptible than the egg stage. The mortality responses of the confused flour beetle adult and the black carpet beetle larva to sulfuryl fluoride were compared by using varying dosages, exposure periods and temperature conditions. Sulfuryl fluoride was more toxic than methyl bromide under all conditions in these tests. Good penetration of a variety of commodities and soils was demonstrated by the superior insect kill obtained from the use of sulfuryl fluoride as compared with methyl bromide.

Sulfuryl fluoride had little effect on the germination of 30 kinds of stored weed and crop seeds. It was injurious to green plants, vegetables, fruits and tubers at moderate dosages. Sulfuryl fluoride was sorbed less than methyl bromide in wheat and other materials. Sulfuryl fluoride seems especially suitable for fumigation of termite infested structures and shows much promise for other uses. Combinations of sulfuryl fluoride with other fumigants may be desirable for solving some fumigation problems.

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[3] "Gow-Mac Gas Master," manufactured by the GOW-MAC Instrument Company, Madison, New Jersey.

Sulfuryl Fluoride—A New Fumigant for Control of the Drywood Termite *Kalotermes minor* Hagen[1,2]

DOANE STEWART[3]

Methyl bromide fumigation for the control of the drywood termite (*Kalotermes minor* Hagen) has become more popular during the last few years because it will completely rid a structure of all *in situ* termites. The increased usage is also associated with the increased incidence of the drywood termite in the warmer coastal regions of the United States (Hunt 1949).

Fumigation methods differ with the type of structure to be treated. Wooden buildings are usually covered with gas impervious tarpaulins, while in masonry structures sealing of the outer doors, windows, and vents with masking tape and laminated paper is common. In commercial practice, methyl bromide is normally introduced into the structure at 2 to 3 pounds per 1000 cubic feet which results in satisfactory control of the termites if the seal is adequate and undisturbed for a period of 18 to 24 hours. However, under these conditions, methyl bromide can cause persistent mercaptan-like odors in several sulfur-containing materials such as rubber, leather, animal hair, and iodized salt. Prior to fumigation, household effects which are obvious troublemakers are removed, but it is not uncommon for some to be overlooked. Consequently, unpleasant odors sometimes do occur. The expanding use of foam rubber in furniture and rubber backings on carpets has further accentuated the problem.

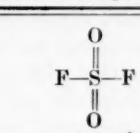
Sulfuryl fluoride is a very promising compound that has emerged from a study designed to find a relatively trouble-free fumigant for structural usage. E. E. Kenaga[4] was the first to report the insecticidal activity of this compound. It is a highly stable, colorless, odorless, nonflammable material. Some of its physical properties are given in the list below. Other properties in connection with its use as a fumigant for termite control are discussed in this paper.

IN VITRO METHODS.—Drywood termites were collected from naturally infested eucalyptus logs near Seal Beach, California. Healthy fourth to seventh instar worker nymphs were confined in suitable containers with a 1-inch square of paper toweling for mechanical support and a source of food. Two replicates of 25 termites each were used in two separate fumigations. The laboratory fumigation chambers were 19.5-liter, wide-mouth, glass jars in which a small tin plate propeller was rotated by a magnetic stirrer for gas agitation. The screw-type lid was coated with a heat-treated epoxy resin; a polyethylene washer lightly coated with silicone grease effected a tight seal. Gas was introduced into the chamber through a $\frac{1}{4}$-inch copper tube soldered into the lid; a vacuum of 50 mm. Hg below atmospheric pressure was created in the chamber to facilitate introduction of the gas from a burette. The termites, after removal from the chambers, were maintained at 21 to 27° C. Mortality counts were taken 5 to 14 days after the exposure period and the results were composited to give the LD 96 to 100.

EXPOSURE-CONCENTRATION.—The concentration of sulfuryl fluoride required for 96 to 100% mortality of the drywood termite for exposure periods of 1 to 24 hours is shown in figure 1. Methyl bromide is included for comparison. Dosage is plotted on the log scale and hours exposure on the linear scale. The two compounds appear to be about equally toxic; however, the development of toxic symptoms is slower with methyl bromide and it is not until approximately 24 hours post-exposure that the toxicity of the two materials appear about equal. After 3 to 5 days post-exposure further toxic effects of the fumigants have disappeared and the termites which have not died continue to live for several weeks or more.

TEMPERATURE-CONCENTRATION.—Temperature will influence the results obtained in structural fumigation to a large degree. This point is illustrated by figure 2 in which temperature is plotted against the concentration of sulfuryl fluoride required for 96 to 100% *in vitro* mortality of the drywood termite. Methyl bromide is included for comparison. Here, again, the dosage requirements of the two compounds are similar, within what is considered a normal temperature range for the drywood termite. However, sulfuryl fluoride is more sensitive to temperature change than methyl bromide, being slightly more toxic above 13° C. and less toxic below this temperature. As the temperature is reduced, the termites in the sulfuryl fluoride treatments became increasingly lethargic so that below 6° C. they are entirely immobile. This effect is not as pronounced in the methyl bromide treatments nor in the untreated control. These data are meaningful from the standpoint that structural fumigations are

Physical properties of sulfuryl fluoride

Structure	
Boiling point	−55.21° C.[a]
Melting point	−136.67° C.[b]
Molecular weight	102.1
Critical temperature	96 ± 20° C.[b]
Liquid density	1.36 g./cc. at 21° C.[c] 1.08 g./cc. at 58° C.[c]
Gas density	3.72 g./cc. at 0° C.[d]
Absolute pressure	235 p.s.i. at 21° C.[c] 540 p.s.i. at 54° C.[c]
Water solubility	0.1% at 10° C.[c]

[a] Determined by S. A. Shrader, The Dow Chemical Company, Midland, Michigan.
[b] Determined by D. R. Stull, The Dow Chemical Company, Midland, Michigan.
[c] Determined by A. P. Hobbs, The Dow Chemical Company, Midland, Michigan.
[d] Handbook of Chemistry and Physics.

PAID PAPER.
[1] Accepted for publication November 14, 1956.
[2] Paper presented, in part, at the Pacific Branch meeting of the Entomological Society of America. Berkeley, California, June 1956.
[3] Agricultural Laboratory, The Dow Chemical Company, Seal Beach, California.
[4] Agricultural Chemical Research Laboratory, The Dow Chemical Company, Midland, Michigan.

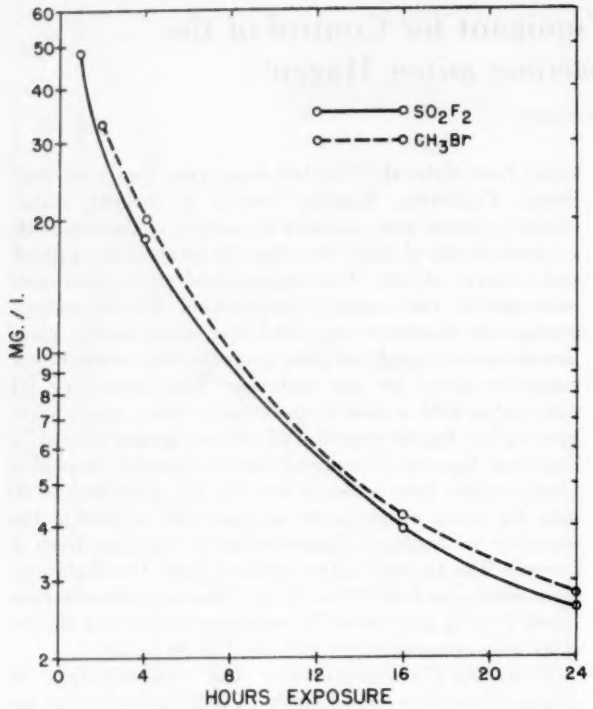


FIG. 1.—Dosage of sulfuryl fluoride and methyl bromide required for 96 to 100% mortality of the drywood termite at various exposures at 21° C.

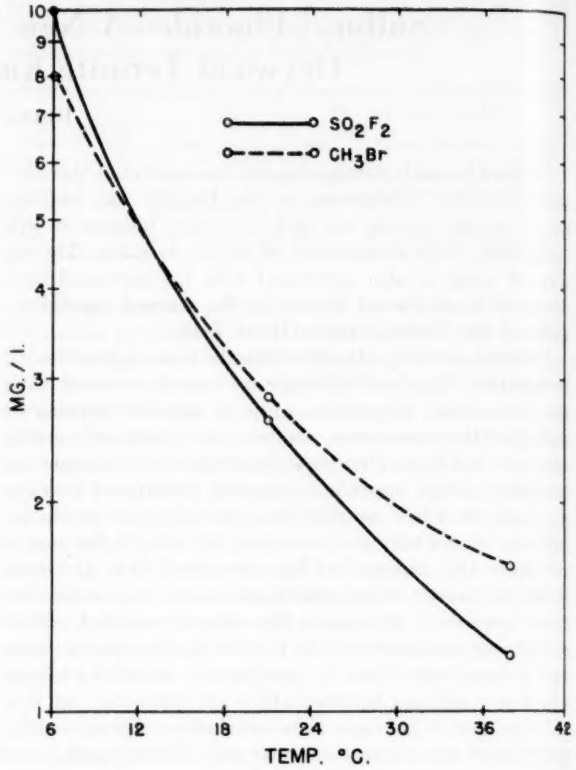


FIG. 2.—Dosage of sulfuryl fluoride and methyl bromide required for 96 to 100% mortality of the drywood termite at various temperatures for 24-hour exposures.

conducted throughout the year and, at least in present practices, all heating equipment is turned off. Thus, the economy of the fumigant is related to the mean temperature during the exposure period.

PENETRATION AND SORPTION.—Sulfuryl fluoride and methyl bromide were compared for their ability to penetrate through sawdust. Three replicates of 33 termites each were confined in screen-topped vials in the bottoms of 25×400 mm. test tubes. Forty-four grams of air-dry, mill-run sawdust[5] were tamped into a column 28 cm. in height over each termite container. The tubes were exposed to the fumigants in fumigation chambers at 21° C. for 24 hours. After the tubes were removed from the fumigant, they were allowed to aerate for 18 hours to represent the condition encountered in practice where the escape of the fumigant is impeded, and there is a post-fumigation effect. The sawdust was then removed, and the termites placed in petri dishes and maintained at laboratory temperatures. Table 1 shows the results of this experiment. The dosages of 2.2 and 2.4 mg. per liter of the respective fumigants were used to give a mortality figure below 100%. It is readily evident from the results that the movement of sulfuryl fluoride was hindered less by the sawdust than was methyl bromide. The two compounds have approximately the same diffusion rate in free air; therefore, it is concluded that sulfuryl fluoride does not sorb or react with wood as readily as methyl bromide.

Table 1.—Comparative penetrability of sulfuryl fluoride and methyl bromide through sawdust as indicated by drywood termite control.

MATERIAL	RATE MG./LITER	PER CENT DEAD 5-DAYS POST-EXPOSURE
SO_2F_2	2.2	94
CH_3Br	2.4	7

CONFINEMENT OF FUMIGANT.—The largest variable in practical fumigation is the effectivity with which the gas is confined within the structure. Although the physical aspects of laboratory experiments can be fairly well controlled, every commercial use of tarpaulins or sealing job will differ in effectivity with each type of structure, location, operator, equipment and weather condition.

The following experiment was conducted in a 500 cubic foot out-of-door structure, designed to measure the effect of incomplete sealing. The structure was an 8-foot cube, constructed of 2″×4″ wooden framework, covered with masonite and sealed by an envelope of 4-mil polyethylene sheeting which was heat-sealed at the seams. The floor of the chamber was moist clay soil which was also used to seal the bottom edges of the chamber.

Holes 1 inch in diameter were drilled at both the top and bottom of two of the side panels giving a total of four holes. In the first treatment the holes remained open, but in the second treatment the holes were closed with rubber stoppers. In both treatments sulfuryl fluoride was dispersed into the air stream of an electric fan at a

[5] Sawdust consisted of fir, pine and redwood collected from lumberyard.

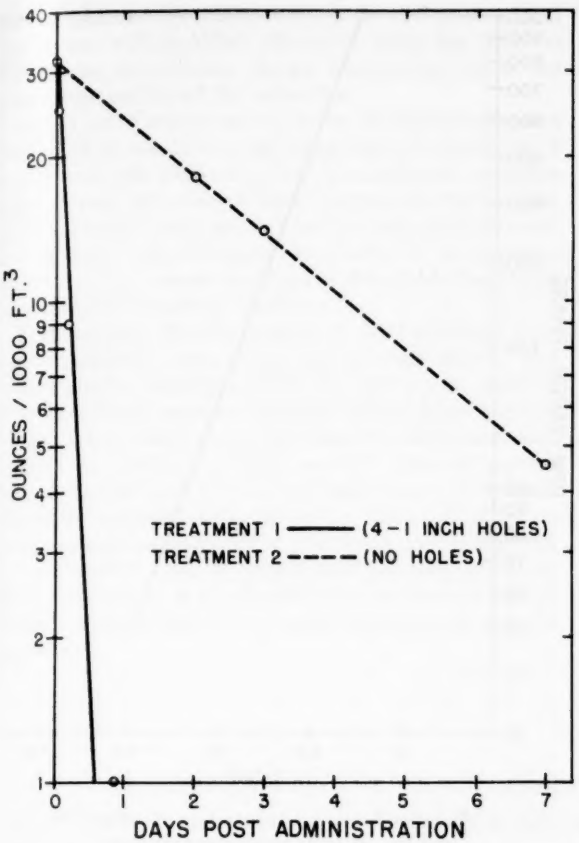


FIG. 3.—Concentration of sulfuryl fluoride retained in a sealed and unsealed fumigation chamber.

concentration of 2 pounds per 1000 cubic feet. There were intermittent winds of 3 to 5 m.p.h. during the course of both treatments. A thermoconductivity unit[6] was used to measure the fumigant concentrations. The results which are plotted in figure 3 show the concentrations of sulfuryl fluoride retained in the sealed and unsealed chambers. Under sealed conditions the concentration dropped from 32 ounces to 24 ounces per 1000 cubic feet in 3 days and to 4.5 ounces in 7 days. These figures are in contrast with those for the unsealed conditions in which the concentration dropped to 9 ounces in 5 hours and after 21 hours the concentration was below the 1-ounce level sensitivity for the equipment.

TARPAULIN MATERIAL.—It has been determined[7] that sulfuryl fluoride at room temperature and at a concentration of 35 mg. per liter penetrates 4-mil polyethylene sheeting at a rate of 6.2 mg. per square meter per hour. Methyl bromide under the same conditions penetrates at the rate of approximately 15.5 mg. per square meter per hour, which is about 2.5 times that for sulfuryl fluoride. It was computed from these figures that during the fumigation of an 18,000 cubic foot structure about 50 grams of sulfuryl fluoride or 125 grams of methyl bromide would be lost through the tarpaulin material. These amounts are a small fraction of the 36 pounds of fumigant that would normally be used on a structure of this size. In addition to polyethylene sheeting, 7-ounce polyvinyl-coated nylon and neoprene-coated nylon have given satisfactory results in several field fumigation trials with sulfuryl fluoride. All three types of tarpaulin material have been extensively used commercially with methyl bromide.

FORCED CIRCULATION.—At the present time structural fumigations are usually conducted without the use of mechanical circulation equipment. Until rather recently most operators considered that the introduction of heat-vaporized methyl bromide into the attic region resulted in a rapid and even distribution of the gas throughout a structure. However, within the past 3 years, measurements of fumigant dispersal by the thermoconductivity cell have proved this hypothesis to be a fallacy. This type of application usually results in a rapid flow of the gas into the lower portions of the structure from which it disperses upward at a slow rate.[8] The rate is sometimes so slow that if the use of tarpaulins or the sealing job is poor the heavy rich mixture will leak out of the structure faster than it will disperse upward. The overall effect is that there may be insufficient toxicant in the upper portions of the structure for complete control of the termites. The gas density of sulfuryl fluoride is only slightly lower than that of methyl bromide (3.40 grams and 3.97 grams per liter at 25° C. respectively). Both of these gases are considerably heavier than air which is 1.18 grams per liter at the same temperature. It is for this reason that there is an initial settling effect of the two fumigants when introduced into free air.

Table 2.—Distribution of sulfuryl fluoride introduced into a stucco garage without mechanical circulation.

LOCATION	OUNCES SO_2F_2 PER 1000 CU. FT. PER ELAPSED TIME			
	2 Min.	15 Min.	90 Min.	24 Hours
Ceiling	7	2	2	4
Center	15	8	22	6
Floor	—	112	42	10

Table 2 shows the distribution of sulfuryl fluoride in a stucco garage in which no circulation equipment was used. The garage was fairly well sealed with masking tape and laminated paper over the double door. The cracks where the mudsill joined the concrete slab floor were no larger than normally encountered. A thermoconductivity cell unit was used to measure the gas concentration through sample tubes located at the ceiling, center, and floor levels of the building. Sulfuryl fluoride was introduced as a liquid onto the floor at the concentration of 20 ounces per 1000 cubic feet. The convection caused by the administration of the gas gave the higher initial concentration at the center and ceiling levels, but the heavy vapors settled rapidly. The data show that the subsequent diffusion upward was not as rapid relatively as was the loss of gas from the bottom of the structure. Although sulfuryl fluoride reached a concentration sufficient to kill all test termites placed at the ceiling level, this dispersion method

[6] "Fumiscope" manufactured by R. K. Hassler, Altadena, California.
[7] S. A. Shrader, The Dow Chemical Company, Midland, Michigan.
[8] Unpublished communication by Karl Hassler, Neil A. Maclean Company, Los Angeles, California.

does not use the gas efficiently nor could control necessarily be expected in a fully partitioned building or one less tightly sealed.

In contrast with the foregoing situation, a fairly well sealed stucco house was fumigated with sulfuryl fluoride which was dispersed into the air stream of a centrifugal fan having a capacity of 3000 cubic feet per minute, which was placed on a bedroom floor. The air stream was directed toward a closet in which there was a small access hole into the attic area. The fan was operated for the first 30 minutes of the fumigation. A thermoconductivity cell unit was used to measure the gas concentrations as in the previous experiment. The distribution of sulfuryl fluoride at various locations in the structure is shown in table 3.

The data clearly show that forced circulation rapidly and effectively disperses sulfuryl fluoride throughout a structure such as a house. In this experiment the gas was lost most rapidly from within the outside wall and the sub-floor areas but, since the gas was evenly distributed early in the fumigation, the loss was proportionately less than if it had not been mechanically dispersed.

DISPERSAL OF FUMIGANT.—Supplementary heat is not necessary to vaporize sulfuryl fluoride before introducing it into a structure. The boiling point is −55.2° C.; thus, at temperatures encountered in practical fumigations (above 45° F.), it evaporates almost as soon as it is released in the liquid phase from the cylinder. This feature offers a distinct advantage over methyl bromide which generally is heated as it is introduced in order to avoid the hazard of marring interior finishes. Sulfuryl fluoride has been used in several structural fumigations in which it was dispersed into the air stream of a fan. There has been no evidence of staining or marring of painted finishes even though the fumigant outlet has been placed as close as 3 feet from finished surfaces. When liquid sulfuryl fluoride is discharged through ¼-inch OD polyethylene tubing the rate of flow is approximately 5 pounds per minute. Consequently, the time required for introduction of this fumigant is considerably less than for methyl bromide.

AERATION MONITORING.—It is necessary for the fumigation operator to have a method to determine whether or not sulfuryl fluoride has been adequately aerated from a structure before occupancy. At present, the halide leak detectors, as used for the detection of methyl bromide, are not adequate for the detection of sulfuryl fluoride. However, until a more simplified method is available, the following method will allow an operator to sample and determine a wide range of concentrations of the fumigant with a fair degree of accuracy.

Table 3.—Distribution of sulfuryl fluoride introduced into a stucco house with mechanical circulation.

LOCATION	OUNCES SO_2F_2 PER 1000 CU. FT. PER ELAPSED TIME			
	5 Min.	30 Min.	60 Min.	21 Hours
Attic	18	14	12	8
Front room	24	18	12	10
Within outside wall	16	—	8	4
Sub-floor	10	12	12	4

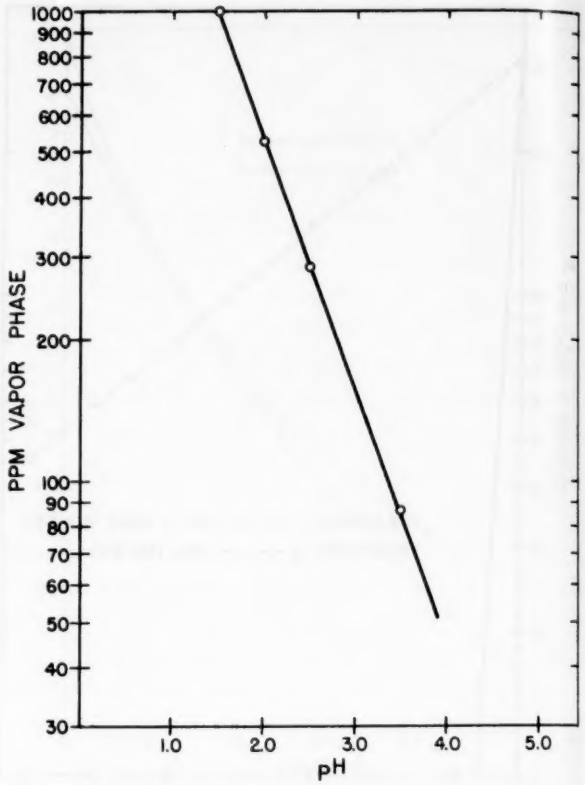


FIG. 4.—Relation between concentration of sulfuryl fluoride and the pH of its combustion products.

A conventional propane halide leak detector[9] with the chimney extended 3 inches, so that the exhaust gases are reduced in temperature, is employed. The operating valve is set to maintain the reaction plate at a dull cherry red because the rate of intake and the combustion of the fumigant is in relation to the rate of flow of the propane and the temperature of the flame. A strip of wide range pH indicator paper[10] is dipped in distilled water and then placed across the top of the chimney so the gases of combustion come in contact with the paper for 10 seconds. The color change in the paper relates the pH to the sulfuryl fluoride concentration. Figure 4 shows the straight line relationship where the pH is plotted on the linear scale and the concentration on the log scale.

Preliminary studies with tagged S^{35} sulfuryl fluoride on several household effects show that it is not as readily retained as methyl bromide. Therefore, safe aeration practices as used for methyl bromide will suffice.

PHYTOTOXICITY.—Sulfuryl fluoride is phytotoxic at concentrations which would normally be used in structural fumigation. At the rate of 1 pound per 1000 cubic feet, it severely injured or killed the following plants in a 500 cubic foot fumigation chamber: Bougainvillaea, Euginia, Nasturtium, Poinsettia, Pyrocantha, Valentia Orange Seedling, Veronica and Viburnum ivy. Until specific tolerances can be demonstrated it must be assumed

[9] "Bernz-O-Matic," manufactured by Otto Bernz Co., Inc., Rochester, New York.

[10] "p-Hydrion paper," Micro Essential Lab., Brooklyn, New York.

that all plants subjected to sulfuryl fluoride in structural fumigation will be killed. However, there has been no damage to ornamentals during fumigations when the plants were outside of the tarpaulins.

ODORS AND CORROSION.—A series of experiments were conducted in the laboratory using sulfuryl fluoride at 2 to 4 pounds per 1000 cubic feet in an attempt to induce odors in and corrosion of many household effects. Also, sulfuryl fluoride was used at 1 to 2 pounds per 1000 cubic feet in eight fully furnished structures. In no instances were there any odors or corrosion detectable that could be attributed to sulfuryl fluoride.

TOXICOLOGY.—Experiments by T. R. Torkelson[11] have shown that both sexes of rats, guinea pigs, rabbits, and female rhesus monkeys tolerated 100 p.p.m. sulfuryl fluoride without apparent adverse effects when exposed 7 hours a day, 5 days a week for 6 months. This conclusion is based on a study of growth, mortality, general appearance, behavior, and the normal appearance of internal organs at autopsy. Although sulfuryl fluoride is by no means as toxic as many of the commonly used fumigants, it is sufficiently toxic to require safe handling practices. For a comparison, it is only one-third as toxic as methyl bromide when measured by acute inhalation of a single exposure.

It has been determined[12] that activated charcoal will absorb two-thirds as much sulfuryl fluoride as methyl bromide; however, experiments[11] with commercially available canisters reveal that their efficiency of sulfuryl fluoride sorption is below that predicted. At 20,000 p.p.m. this type of canister is not reliable for more than 10 minutes and at concentrations of 200 p.p.m. the limit of reliability is 1 hour.

SUMMARY.—Sulfuryl fluoride, a new structural space fumigant for the control of the drywood termite, *Kalotermes minor* Hagen, is discussed. This compound is compared with methyl bromide which is extensively used for controlling drywood termites. Advantages of sulfuryl fluoride are its nonflammability, penetrating ability, ease of application, non-corrosiveness, and lack of malodorous reactions. Commercial use of tarpaulins and sealing practices with forced gas circulation are satisfactory for sulfuryl fluoride fumigation. Precautions for its usage are given.

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[11] T. R. Torkelson, The Dow Chemical Company, Midland, Michigan.
[12] A. Hobbs, The Dow ChemicalCompany, Midland, Michigan.

Levels of DDT-Dehydrochlorinase during Metamorphosis of the Resistant House Fly[1]

HERBERT H. MOOREFIELD[2] and C. W. KEARNS, *University of Illinois, Urbana*

The development of a tolerance for the chlorinated hydrocarbon insecticides by various species of insects appears to be a very complex phenomenon. At the present time, the best documented phase of tolerance to DDT [1,1,1-trichloro-2,2,bis (*p*-chlorophenyl) ethane] is the role of detoxication. In 1950 it was reported by two groups working independently that the resistant house fly (*Musca domestica* L.) could degrade DDT to a nontoxic metabolite DDE, [1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene], (Sternburg *et al.* 1950, Perry & Hoskins 1950). It was later shown that the metabolic process was enzymatic in nature (Sternburg *et al.* 1953). In subsequent studies involving 15 strains of flies, it was found that the detoxifying enzyme, DDT-dehydrochlorinase, could not be detected in susceptible flies but was demonstrable in resistant fly populations in levels correlating with the degree of tolerance expressed by topically determined LD_{50}s, (Sternburg *et al.* 1954). Investigations on the mode of action of certain nontoxic DDT synergists confirmed the importance of metabolism in resistance by showing that a synergist potentiating DDT proves a toxic combination to the resistant fly because of the ability of the added compound to inhibit the detoxifying enzyme (Moorefield & Kearns 1955). The current study was undertaken to determine if the enzyme, DDT-dehydrochlorinase, is present throughout the life cycle of the resistant house fly and to quantitatively trace it through the stages of metamorphosis.

MATERIALS AND METHODS.—The flies used in this investigation were reared in the standard National Association of Insecticide and Disinfectant Manufacturers medium. Origin and maintenance of the various strains have been previously described (*ibid*). Eggs, larvae and pupae were all thoroughly washed and blotted dry before homogenization.

Enzymatic assays were performed on homogenates consisting of 200 individuals (irrespective of stage of development) ground in 20 ml. of cold, distilled water. Insoluble parts were removed by centrifugation or filtration through cheesecloth. The reactions were carried out in single arm Warburg vessels containing 4 mg. of substrate. The DDT was crystallized through air evaporation of an acetone solution onto glass beads (15 to 60 microns in diameter) directly in the body of the flask. Two ml. of homogenate, 0.5 ml. of 0.137 molar phosphate buffer, pH 7.4. were delivered and the vessels were shaken under nitrogen for 15 minutes while equilibrating with the bath temperature, 37° C. Ninety-minute reactions were run, measured from the time of introduction of 3 mg. glutathion from the side arm. Quantitative determinations of the mixed substrate, DDT, and product, DDE, were performed after treatment of each vessel with 3 ml. concentrated sulfuric acid and extraction with

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[2] Now Entomologist, Carbide and Carbon Chemicals Co. Fellowship, Boyce Thompson Institute for Plant Research, Inc., Yonkers 3, New York.

cyclohexane. Ultraviolet absorbance at 241 and 260 mμ. was measured with a Beckman DU spectrophotometer and standard calculations for a two component system applied. These procedures have been reported in detail (Sternburg *et al* 1954). Corrections for endogenous ultraviolet absorbance on untreated control homogenate samples were made concurrent with each assay.

A minimum replication of two successive generations of each strain of flies were analyzed and each individual determination was performed in duplicate. The data presented is an average of the figures derived from assays of a single generation.

RESULTS AND DISCUSSION.—The comparative assays performed in this study were designed to measure the total DDT-dehydrochlorinase activity occurring throughout the various life history stages of the resistant house fly and to express the results in terms that would reflect the levels of enzyme in a single fly. To minimize the interfly variation, samples of 200 individuals of each stage were homogenized in a constant volume; conditions of preparation and assay were standardized; and rigid experimental controls observed. Relative concentration of the detoxication enzyme during growth and development of the resistant fly (S strain) are graphically depicted in figure 1.

Significant amounts of DDE were not produced by homogenates of eggs or 24 hour larvae, even in more concentrated preparations containing as much as 3 gm. of eggs and 6 gm. of larvae in the same volume. Whether the enzyme is truly absent in these stages or merely undetectable by the methods used was not established. Although the catalytic dehydrochlorination mechanism increases rapidly, paralleling larval growth, at the time of pupation a striking reduction in the total enzymatic activity occurs. This general pattern was observed in three strains of resistant flies (table 1), and the following experiment was designed to afford strict random sampling. Before any pupation took place in a given jar, all of the larvae were separated from the media, placed in a flat pan, and representatives counted out and frozen. The early, red-colored pupae were removed as they reached this stage of development. These were pooled in the deep freeze. After all of the individuals had been collected, representative samples were assayed on both larvae and pupae. The freezing process did not alter the level of enzymatic activity.

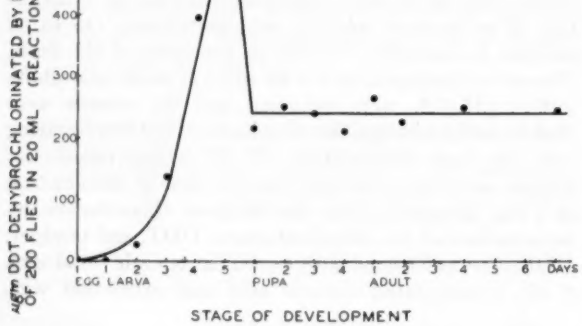

FIG. 1.—Relative Levels of DDT-dehydrochlorinase during development of the resistant house fly.

Table 1.—Comparative DDT-dehydrochlorinase activity in different strains of resistant house flies.

STAGE OF DEVELOPMENT (EXPRESSED IN DAYS)	μGM. DDE PRODUCED IN 90′ BY HOMOGENATES OF 200 FLIES IN 20 ML. S Strain[a] [selected with DDT]	DMC Strain[b] [Selected with DDT+ (p-ClC_6H_4)$_2$$COHCH_3$]	Syn. Strain[c] [Selected with DDT+ (p-ClC_6H_4)$_2$$CHCH_3$+ ($p$-$ClC_6H_4$)$_2$$CHCl$]
Larva 2	20		45
3	140	235 (3.1 gm.)	275 (2.9 gm.)
4	400 (3.4 gm.)[d]	420 (3.6 gm.)	
5	570 (4.6 gm.)	630 (4.5 gm.)	695 (4.6 gm.)
6		715 (5.3 gm.)	760 (5.2 gm.)
Pupa 1	210 (4.2 gm.)	390 (4.4 gm.)	500 (4.8 gm.)
2	260 (4.1 gm.)		
3	245 (4.5 gm.)	365 (4.5 gm.)	490 (4.1 gm.)
4	210 (4.2 gm.)		
Adult 1	270 (3.3 gm.)	360 (3.4 gm.)	515 (3.4 gm.)
2	230 (3.5 gm.)	360 (3.7 gm.)	
4	255 (3.8 gm.)	385 (3.6 gm.)	530 (3.8 gm.)
8	240 (3.6 gm.)		500 (3.9 gm.)

[a] Obtained from Dr. W. N. Bruce, State Natural History Survey, Urbana, Illinois.
[b] Obtained from Dr. R. W. Fay, U.S.P.H., Savannah, Georgia.
[c] Obtained from Dr. R. B. March, Citrus Experiment Station, Riverside, California.
[d] Total wet weight of population sample.

An observation similar to the decrease in DDT-dehydrochlorinase activity found at the time of house fly pupation has also been described for a lepidopteran (Way 1954). In a study correlating the body weight of larval *Diataraxia oleracea* L. with the tolerance to various contact insecticides, it was found that resistance to DDT progressively increases up to the fifth or sixth day of development when a sudden decrease occurs coinciding with the cessation of feeding and beginning of prepupa formation. Such a process could be explained in both the house fly and tomato moth if a major site of detoxication were located in those tissues which are early histolyzed at the time of pupation. Indiscriminate degeneration of the protein constitutents of a given locus or organ may well partially inactivate a systen involving metabolism of a toxicant such as DDT. Evidence of a detoxication site in the fly cuticle (Sternburg & Kearns 1950, Tahori & Hoskins 1953) together with the demonstration of proteases in the insect moulting fluid (Passonneau & Williams 1953) could well support such a proposal.

The quantity of DDT-dehydrochlorinase expressed in the early pupae is maintained at a near constant level for the remainder of the resting stage and throughout the adult life of the fly. Adults reared on sugar-water experience a gradual decline in total protein (measured by Kjeldahl determination of protein nitrogen), as would be expected, but this is not reflected in the concentration of detoxifying enzyme (table 2). Likewise, flies receiving a milk diet exhibit no increased resistance as measured by enzyme activity.

The finding that larvae of a given strain are more tolerant of DDT than adults of the same population is not new (Sternburg & Kearns 1950), but the fact that this can be correlated with corresponding changes in the level of DDT-dehydrochlorinase presents additional evidence in support of the significance of detoxication in DDT re-

Table 2.—**Effect of diet on level of DDT-dehydrochlorinase in adult resistant flies (DMC strain).**

DAYS AFTER EMERGENCE	μGM. DDE PRODUCED IN 90′ BY HOMOGENATES OF 200 FLIES IN 20 ML.	
	Sugar-Water Diet	Milk Diet
1	370	365
2	370	335
3	360	375
5	365	390
7	360	365

sistance. This difference in tolerance between the two life stages has been shown to be independent of penetration factors as both resistant and susceptible larvae absorb DDT at rates comparable to those for the adults (Tahori & Hoskins 1953).

Several groups of workers have reported that the heterogeneity in degree of resistance among individual flies of a given population is manifested in the timing of metamorphic events. Investigations by Pimentel *et al.* (1951), pointed out that the length of the larval period of DDT-resistant strains appeared to be related to susceptibility. Correlations could be drawn between a longer larval period and a higher degree of resistance within a population. Later Decker & Bruce (1952) found that by selecting flies on the basis of early and late emergence, two sub-populations could be established which varied in resistance as measured by topical applications. The more susceptible flies were the first to emerge. More recently, extensive experiments in which selection of early and late developed pupae were made and adults of these insects inbred for a series of successive generations has demonstrated that valid differences may exist in flies so selected (McKenzie & Hoskins 1954). Resistance to DDT in the resulting early pupating group decreased, whereas, tolerance of the late pupating group increased without exposure to the toxicant.

Further indication of the significance of the metabolic role in insecticide resistance could be documented if levels of the enzyme, DDT-dehydrochlorinase, in flies selected at the time of pupation and emergence were to parallel the observations described. The relative activity of equivalent homogenates of flies selected at both these stages did indeed reflect differences in capacity to enzymatically dehydrochlorinate DDT (table 3). Early pupating individuals, as well as those emerging first, contained lower detoxifying potential as expressed by *in vitro* activity than did those developing later. No attempt was made to correlate these differences with the sex of the emerging adults.

Table 3.—**Comparative levels of DDT-dehydrochlorinase within a resistant fly population selected at pupation and adult emergence (DMC strain).**

STAGE	DIVISION OF POPULATION	μGM. DDE PRODUCED IN 90′ BY HOMOGENATES OF 200 FLIES IN 20 ML.
Pupation	Early pupation	370
	Intermediate	395
	Late	440
Adult emergence	Early emergence	270
	Intermediate	340
	Late	375

SUMMARY.—The detoxication enzyme, DDT-dehydrochlorinase, quantitatively increases during the larval period of the DDT resistant house fly. A marked reduction in the amount of enzyme, occurring at the time of pupation, may decrease the total activity up to 50%. The resultant level is maintained throughout the pupal and adult life. Protein free diets or those supplemented with milk do not significantly affect this level in the adult fly.

Assays on fractions of resistant fly populations selected at the time of pupation or adult emergence indicate that individuals transforming earlier at these stages contain less DDT-dehydrochlorinase than those pupating or emerging later.

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Control of San Jose Scale on Fruit Trees During the Prebloom Period of Pears[1]

H. P. Lanchester and F. P. Dean, *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

Parathion has been reported to control many species of scale on various fruit trees. Boyce (1950) reported it highly toxic to six species infesting citrus trees, and ineffective against one, the soft scale (*Coccus hesperidum* L.). Ewart *et al.* (1951) reported that sprays of parathion to citrus trees in the late winter and early spring gave commercial control of citricola scale. O'Neill (1951) used it against San Jose scale on prunes at the green-tip stage of bud development, in the first cover spray, and just before the appearance of the crawlers. The first and third times of application were the most satisfactory. O'Neill & Hantsbarger (1952) reported that in 1948 parathion gave control of all stages of San Jose scale. They recommended a prepink application, since thorough coverage is easier before the trees are in full foliage. The Washington Agricultural Experiment Station (1956) recommends a prepink spray of 1 pound of 25% parathion wettable powder per 100 gallons, since this time gives the best opportunity to obtain enough coverage for scale control.

The grower is better able to determine the green-tip stage of the prune buds than the before-crawler stage of the scale, and thorough coverage is simpler on a bare tree. But the term "prepink" applies to a stage when the buds show the first color and, in apples and pears, have not separated in the cluster. It may last but a few days and occurs at a time of the year when spraying conditions are seldom good. If the treatment is delayed until bloom, bees may be killed. Growers have been reluctant to wait for the prepink stage, and have asked about using parathion earlier. Growers and research men told of applications in late winter and early spring which varied from no control to excellent. Was there a time factor?

1953 Tests.—In the spring of 1953 a series of tests were made on pear trees to determine the period of time during which a prebloom spray of parathion would control the San Jose scale. Entire trees were sprayed at each application at the rate of 1 pound of 25% parathion wettable powder per 100 gallons of water. The first application was made on February 26, and others at 5- or 6-day intervals until April 7.

At the time of the first application the scales and buds appeared dormant. On March 4 a few of the scales showed signs of adding to their covering. This scale activity increased slowly until the March 19 application, which was the first to give an increase in control, but not enough to promise commercial freedom from injury. On March 24 the fruit buds were greatly swollen and their scales covered less than half of the bud. Applications at this time and on March 30 and April 7 gave good control. The condition of the buds on these dates is shown in figure 1.

Counts of living scales were made just before each application. Post-treatment counts were delayed until the scales had been active at least a month and the applications had been made 30 days. The percentages of scales alive at these before- and after-spraying counts are given below.

Date	Before Spraying	After Spraying
February 26	72	30
March 4	70	32
9	80	26
13	81	23
19	81	11
24	77	4
30	76	3
April 7	69	2

The temperatures following the later applications were no higher than those following the earlier ones. The highest temperatures of the period were in the first days of March. Clearly the increased kill from the later applications was not due to greater insecticidal activity but to the increased activity of the scales.

1954 Tests.—In the spring of 1954 tests were made for the control of San Jose scale on pears with malathion and demeton. Parathion was used for comparison. The dosages per 100 gallons were malathion (57% EC) 13 ounces, demeton (21% EC) 24 ounces, and parathion (25% WP) 1 pound. Applications were made on March 8, when the fruit buds were beginning to show growth, and on April 2, 9, and 16. Weather conditions following the March 8 application were such that no further growth was apparent for nearly a month. The pear trees were in full bloom on April 26. The trees were part of an abandoned orchard and no additional sprays were applied during the year.

Counts to determine scale mortality were made at the time the sprays were applied and again from May 14 to June 3, and to determine the infestation on the fruit on August 11. At the time of spraying 16% of the scales were dead, the range being 13 to 18%. In the post-treatment counts the per cent dead ranged from 23 on the unsprayed trees to 35 to 46 on trees sprayed with malathion, 22 to 32 on those sprayed with demeton, and 96 on those sprayed with parathion. On August 11 ten fruits were taken from each tree and used for obtaining the last record. The maximum number of scales on a pear from an unsprayed tree was 328 and on one from a sprayed tree 176. The average number of scales on pears from trees sprayed with parathion was 2, on those from trees sprayed with demeton 40, with malathion 48, and on the unsprayed checks 86.

With the tremendous scale population present in this group of pear trees, the results from the parathion treatment 10 days before bloom indicate a high degree of control. The malathion and demeton treatments did not provide control, but they did have more effect on the scale population than the May and June counts indicated.

1955 Tests.—The question arose whether a similar parathion treatment so timed would be equally effective in controlling the scale on other hosts. Accordingly, parathion (25% WP) at the rate of 1 pound per 100 gallons was applied to scale-infested peach trees on April 13, 3 weeks before bloom. Diazinon (25% WP) at the

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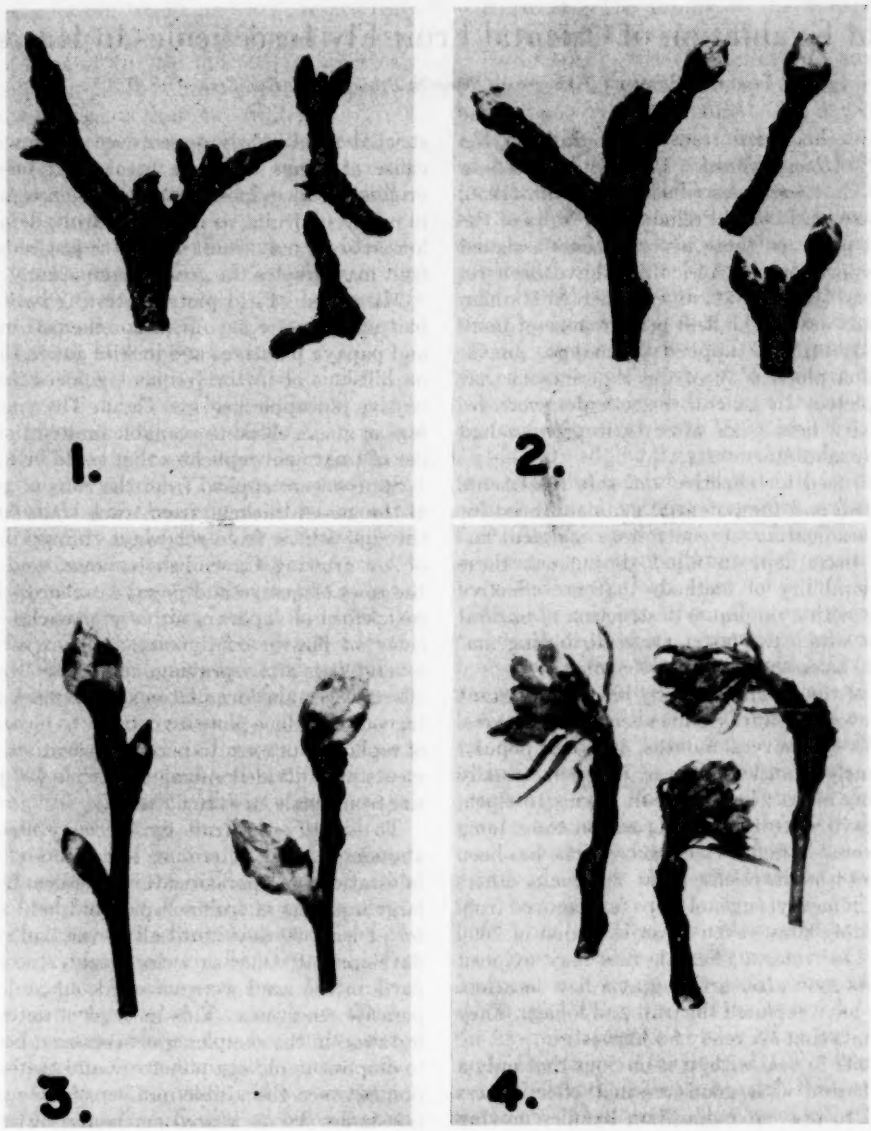

FIG. 1.—Condition of pear buds in 1953 on: 1, February 26; 2, March 24; 3, March 30; and 4, April 7.

rate of 2 pounds per 100 gallons was also included in the experiment. The test was repeated on June 3, a month after bloom. when the peaches were in the late shuck-fall stage.

The post-treatment counts were made on June 6 and 28. The results showed a 98% mortality of San Jose scale on peach trees due to parathion applied in the pre-bloom period of pears, and 93% due to a similar parathion treatment 7 weeks later. The Diazinon treatments, on the other hand, showed 77% mortality due to the early treatment and 98% due to the late shuck-fall treatment.

CONCLUSIONS.—It may be concluded from these tests that parathion may be used for the control of San Jose scale on any tree fruit host in the period 2 weeks prior to the pink bud stage of pear trees. At this time the aphids have hatched and the pear psylla is active, so that these pests may also be controlled by such an application. Malathion, demeton, and Diazinon at the concentrations used did not provide sufficient control when used in the delayed-dormant period.

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Field Evaluation of Oriental Fruit Fly Insecticides in Hawaii[1]

LOREN F. STEINER, *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*[2]

The expanded cooperative research program on the oriental fruit fly (*Dacus dorsalis* Hendel) initiated in Hawaii in 1949, has been described by Carter (1950, 1952) and Christenson (1953). Preliminary results of the chemical-control phase of these investigations assigned to the U. S. Department of Agriculture have been recorded by Steiner (1952, 1954) and Steiner & Hinman (1952). This report covers the field performance of more than 180-spray treatments applied to mango, guava, papaya, or banana plots in 30 of the experiments that have been completed. In general, insecticides were selected for extensive field trials after their promise had been indicated by laboratory tests.

Both the local need for effective and safe treatments for fruit fly control and the potential mainland need for procedures for eradicating or controlling incipient infestations have been kept in mind throughout these studies. The desirability of methods that are effective against fruit flies with a minimum destruction of natural enemies in areas where the latter are contributing materially to control has also been fully recognized.

The behavior of the oriental fruit fly has an important bearing on the development of chemical control measures. Adult flies may live for several months, and their populations are extremely transient. Large numbers usually move into fruiting areas when the fruit begins to ripen, and they may leave when the bearing season ends. Long distances are covered in flight. One marked male has been recovered 24 miles from its release point, and many others have been taken in methyl eugenol traps far removed from any known breeding sites—even at an elevation of 7600 feet on Mauna Loa volcano. Female flies may oviposit in available fruits soon after arriving at a new location, or not until they have explored the fruit and foliage. They usually prefer fruits that are ready for harvest.

With such habits to deal with, it is obvious that only a quick-acting toxicant with good residual effectiveness can be expected to prevent oviposition by flies moving into a treated area. It is also apparent that insecticides without residual action would be useful chiefly against isolated populations not subject to replacement by flies migrating into treated plots. The fact that many of the preferred hosts must be harvested continuously for several weeks imposes a limitation on the type or concentration of insecticide that can be used safely, since for these hosts it is not practical to have a prolonged interval between spraying and picking.

In the Hawaiian Islands the oriental fruit fly has a sufficiently wide range of hosts within flight range to insure continuous reproduction. Such hosts may include immature fruits, mature green and ripe fruits still attached to the plant, or fruits that have fallen to the ground. Even produce in markets or in transit may be attacked by the flies. The injury varies according to the type of fruit, but usually consists of breakdown of tissues and internal rotting associated with the maggot infestation. When eggs are inserted into hosts in which few or none of the young larvae can develop, the injury is restricted to unsightly appearance and lowered grade because of stings or tissue breakdown because of decay originating at oviposition sites. When eggs are deposited in newly set fruits, as in passion fruit, deformity and calloused areas may result as growth proceeds, or the young fruit may drop to the ground prematurely.

METHODS.—Field plots for testing both bait and nonbait sprays were set up in commercial mango, banana, and papaya plantings and in wild guava thickets growing on hillsides or in the frequent gulches that transect extensive pineapple fields on Oahu. The size of fruit plantings or guava thickets available limited the size and number of treatment replicates that could be located in them.

Sprays were applied from the rims of gulches in some of the guava thickets, from truck trails cut or bulldozed through others, from roadways through irrigated blocks of low-growing Cavendish bananas, and from between the rows of mango and papaya orchards. Because of the toxic effect of vapors or airborne particles of some insecticides on flies in contiguous check or sprayed plots for several days after spraying, or the repellent or attractive effect of certain formulations, it was not always advantageous to reduce plot size in order to increase the number of replicates or even to permit replication. The 180 treatments were divided among more than 600 plots ranging in size from single trees to 65 acres.

To identify the fruit fly species present, assess their abundance, and determine the effects of treatments on infestations and parasitization, it was necessary to collect large numbers of fruit samples and hold them over sand for at least 30 days until all larvae had completed their development. Once or twice weekly the larvae and puparia in the sand were screened out and held for fly or parasite emergence. This method of determining the infestation in the samples was necessary, because flies tend to oviposit in old egg punctures and there is little correlation between the visible number of sites and the infestation index (larvae reared per pound of fruit). Moreover, without peeling the fruit it is impossible to find all fresh punctures or to determine whether eggs have been deposited in them. Large samples are needed for reliable estimates of the infestation index because of the great variation in numbers of eggs deposited and hatching in one fruit (Steiner & Hinman 1952).

A high degree of correlation between the number of larvae per pound and the percentage of individual fruits infested became evident after many gauvas and mangoes had been held individually. With picked guavas indices of 1.8, 9, and 42 larvae per pound were associated with indi-

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vidual fruit infestations of 10, 50, and 90%, respectively. Picked mango indices of 3, 9, and 30 larvae per pound indicated infestations of 10, 25, and 50%, respectively. These relationships provided another practical means of evaluating results of tests on these two fruits.

Usually from one to three samples were obtained from each treatment replicate one to three times weekly. Average infestations were determined for specific intervals after each spray.

Pretreatment infestation data sometimes provided the only basis for control evaluation. Infestations in untreated plots as large as 3 acres were often depressed by sprays applied to adjacent plots. For this reason, control estimates were likely to be very conservative.

Most of the infestation data presented herein were derived from picked fruit. Picked mature-green guavas may produce as much as 50% more larvae than picked ripe fruit, and the latter twice as many as ripe guavas that have fallen to the ground. Concussion, more rapid breakdown, and pathogenic bacteria or fungi appeared to be responsible for increased egg and larval mortality in the fallen guavas. Guavas were picked at random from the plots, or from marked trees within the plots. In the latter instance on the date of sampling all ripe fruit was removed from the sample trees only. This practice tended to concentrate fly attack within sample sites toward the end of sampling intervals, when spray residues were least effective.

Mangoes of the same quality and stage of ripeness as those being marketed were picked and sampled in competition with regular orchard picking crews, usually three times weekly.

In banana tests bunches in the full three-quarters grade (usually three in each plot replicate) were marked and allowed to ripen while still attached to the plants. All fingers showing color were removed each time samples were taken. Since bananas are normally harvested before color break occurs and before they are attractive to fruit flies, this procedure concentrated fly attack on the few ripe fingers present on the sample bunches, thus putting the experimental insecticides to unusually severe tests.

When papayas were used, the insecticides were applied to single trees or very small plots, and all fruits showing color were removed on each sampling date. Since the mature-green fruit that is normally harvested is only slightly attractive to fruit flies, this procedure also tended to concentrate fly attack towards the end of sampling intervals when some of the fruit on sample trees had reached a stage especially susceptible to attack.

The length of the harvest or ripening period limited the number of sprays that could be applied. Whenever possible, at least 3 or 4 applications were made at intervals of 1 to 4 weeks, but usually 2. Concentrate sprays were applied with a mist blower at rates from 4 to 30 gallons per acre. This blower was modified several times, finally by adding a propeller-type agitator and a 2-piston high-pressure pump discharging 4 gallons per minute. Semiconcentrate sprays of 40 to 100 gallons per acre were applied with a hydraulic sprayer at a pressure of 400 to 600 p.s.i., usually through 2 to 6 nozzles on fixed booms. Dilute sprays up to 600 gallons per acre were applied similarly or through single-nozzle guns manually operated from the sprayer or from the ground.

RESULTS.—The results of the field experiments are given in table 1, parts I and II.

Bait Sprays.—The importance of enzymatic protein hydrolysates in the diet of the oriental fruit fly has been pointed out by Hagen (1953). The development of bait-spray formulas utilizing these and other protein hydrolysates, including a partially hydrolyzed enzymatic form and an acid hydrolysate (Steiner 1952, 1954, 1955b), has made it possible to obtain excellent control of the oriental fruit fly with parathion and malathion at low application rates.

In 13 of 21 tests with such bait sprays in which parathion was used at rates of 1/5 to 2 pounds per acre, the control ranged from 93 to 100% throughout the 2-week period following each spray. When used in nonbait sprays without protein hydrolysates at rates below 2½ pounds per acre, parathion gave less than 90% control in 11 of 14 tests. By adding the protein hydrolysate it was possible to reduce the amount of insecticide by at least 50% and obtain control without excessive residues on ripening fruit. Residues never exceeded 2 p.p.m. a few hours after applications of 2 pounds or less of parathion per acre and they were rarely more than 0.5 p.p.m. after 2 to 4 days.

Malathion at ½ to 1 pound per acre gave 65 to 96% control in 11 experiments when supplemented with protein hydrolysate. In other tests enzymatic protein hydrolysate-malathion bait sprays have given practical control of both the oriental fruit fly and the melon fly in situations where these flies have caused heavy losses of passion fruit (Steiner 1955a). Such sprays are currently being recommended in Hawaii for control of fruit flies attacking mango, guava, and passion fruit (Steiner 1954). The recommended formulas do not contain sugar, which was found to reduce their effectiveness against the melon fly.

Dieldrin was the only chlorinated hydrocarbon tested that gave promising results when used in a protein hydrolysate bait spray. The performance of DDT, lindane, aldrin, and Dilan was not improved by the addition of the hydrolysate with or without sugar. The addition of sugar to either parathion or DDT wettable powder did not significantly increase control. Bait sprays containing tartar emetic and raw sugar were less effective than the protein hydrolysate-organic phosphorus combinations, particularly after the first week of weathering.

Because of their attractiveness to the fruit flies, the protein hydrolysate bait sprays were generally applied with less attention to complete coverage than were nonbait sprays. Many applications were made from only one side of a tree row, or the sprays were wind-driven or blown out over guava gulches up to 150 yards wide. Some applications were made with boom-equipped sprayers that directed the spray through the trees in one to three narrow bands. Lower application and material costs and less danger from excessive residues are definite advantages of the bait sprays, in addition to their generally high standard of performance.

Because less toxicant and less thorough coverage of fruit were needed, bait sprays appeared to be less destructive to the important parasite *Opius oophilus* Fullaway. Parasites, principally this species, constituted 57 to 79% of the total emergence from pupae and larvae recovered from unsprayed guava samples from 1950 to

Table 1.—Results of tests with bait sprays and nonbait sprays against the oriental fruit fly in Hawaii, 1950–55.

Insecticide Formulation and Pounds of Toxicant or Additive per Acre[a]	Host Sprayed	Number of Replicate Plots	Acres per Plot	Number of Sprays	Type of Spray[b]	Number of Larvae per Pound in Checks	Per Cent Mean Control[c]
Part I: Bait sprays							
Parathion (25% WP)—							
2+PH 2+sugar 20	Guava	4	0.05	3	D	6	99
2+YH 1+sugar 5	Mango	1	7.5	3	C	5	98
1.5+PH 0.5+sugar 6	Mango	2	1.0	4	C	13	87
1+PH 2+sugar 10	Guava	5	0.01	6	D	5	81
1+YH 1+sugar 5	Mango	1	7.5	3	D	2	100
	Mango	3	3.0	3	C	4	97
	Guava	1	3.0	2	C	2	97
1+YH 1	Mango	1	7.5	3	D	8	99
1+PHYP 1	Mango	1	7.5	3	C	2	100
0.75+PHYP 1	Guava	3	3.0	3	C	9	76
0.75+SB 1.5	Guava	4	3–6	2	C	8	81
0.6+PH 1.5+sugar 12	Papaya	5	0.01	3	C	14	64
0.6+PH 0.75+sugar 7.5	Banana	1	1.0	5	C	14	93
0.5+PHYP 2	Mango	1	7.5	3	D	5	98
0.5+YH 1+sugar 5	Mango	1	6.5	3	C	9	93
0.5+YH 0.75+sugar 3	Guava	3	3.0	3	C	16	84
0.5+YH 0.75	Guava	3	3.0	3	C	16	94
0.5+YH 0.5+sugar 2.5	Guava	3	3.0	3	C	12	96
0.25+PH 0.5+sugar 5	Guava	2	5.0	3	C	13	93 (91)
0.25+YH 0.5+sugar 2.5	Guava	1	6.0	3	C	20	73
0.2+PH 0.3+sugar 8	Guava	1	5.0	2	D	13	81
1.5+sugar 5	Banana	3	.2	2	C	128	52
0.4+sugar 48	Banana	3	.16	4	D	105	13
Malathion (25% WP)—							
1+SB 5	Mango	1	10.0	4	C	32	94
1+SB 2	Mango	1	7.5	3	C	8	75
	Guava	1	7.0	4	C	20	88
1+PHYP 1	Mango	1	7.5	4	C	9	86
1+LH 1	Mango	1	7.5	4	C	12	96
0.75+PHYP 1	Guava	2	4.0	3	C	9	89
	Guava	3	3.0	3	C	9	65
0.75+LH 1	Guava	2	4.0	3	C	4	74
0.75+SB 1.5	Guava	4	3–6	2	C	6	79
0.75+SB 1	Guava	3	3.0	3	C	10	77
0.5+YH 1	Mango	1	7.5	5	C	24	94[d]
Aldrin (25% Em) 1+YH 1+sugar 5	Guava	1	3.0	2	C	2	7
DDT—							
(75% WP) 1+YH 0.75	Guava	3	3.0	3	C	16	87
(60% Em) 3+sugar 7.5	Banana	1	1.0	4	C	14	71
Dieldrin (50% WP)—							
0.75+SB 1	Guava	3	3.0	3	C	9	87
0.5+YH 0.75	Guava	3	3.0	3	C	16	94
Dilan (25% WP) 0.75+YH 0.75	Guava	3	3.0	3	C	19	81
Lindane (25% WP) 0.25+YH 0.5+sugar 2.5	Guava	1	6.0	3	C	20	21
Tartar emetic 3+sugar 10	Guava	3	3.0	3	C	8	76
	Guava	4	3–6	2	C	8	44
Part II: Nonbait sprays							
Aramite (15% WP) 2.5	Papaya	5	0.10	3	C	14	0
EPN (25% WP)—							
6	Guava	4	.01	2	D	2	78
2.2	Banana	3	.16	4	D	20	96
	Banana	3	.16	4	D	105	56
1.75	Guava	6	.33	2	D	13	77 (71)
1.5	Banana	4	.25	4	C	14	67
1.4	Guava	3	.33	4	D	3	90
1.25	Banana	3	.20	2	C	128	61
Malathion (25%) WP—							
6	Guava	4	.01	2	D	2	80
5	Guava	4	.05	3	D	6	99
1	Guava	1	7.5	4	C	20	36
0.6	Papaya	5	.05	3	C	14	2
Metacide (32% Em)—							
2	Guava	5	.05	6	D	5	85
1	Guava	3	.10	3	C	16	90 (66)
Parathion (25% WP)—							
24	Guava	3	.04	1	D	7	88[e]
6	Guava	4	.01	2	D	2	90

Insecticide Formulation and Pounds of Toxicant or Additive per Acre[a]	Host Sprayed	Number of Replicate Plots	Acres per Plot	Number of Sprays	Type of Spray[b]	Number of Larvae per Pound in Checks	Per Cent Mean Control[c]
5	Guava	4	.05	3	D	6	98
3	Mango	2	.05	3	C	12	93
2.5	Mango	3	3.0	3	C	4	95
2.2	Banana	3	.16	4	D	20	97
	Banana	3	.16	4	D	105	68
2	Guava	5	.33	6	D	5	98
1.75	Guava	6	.33	2	D	13	81 (75)
	Guava	3	.33	4	D	3	98
1.5	Banana	4	.25	4	C	14	80
	Banana	4	.25	4	D	14	79
1.25	Banana	3	.20	2	C	128	40
1	Guava	3	3.0	3	C	7	82
	Guava	3	.10	3	C	16	79 (67)
	Guava	4	.01	1	D	104	65
0.8	Guava	3	.33	4	D	3	58
0.6	Papaya	5	.01	3	C	14	64
0.4	Guava	1	5.0	3	C	16	54
Pyrolan (50% Em)—							
3	Mango	2	.5	4	C	13	95
2	Guava	5	.05	6	D	5	83
Demeton (in Systox)—							
(23% Em) 20	Guava	3	.04	1	D	11	97[f]
10	Guava	3	.04	1	D	11	81[f]
(32% Em) 2	Guava	4	.05	3	D	6	98
	Guava	5	.01	6	D	5	97
	Guava	3	.10	3	C	16	94 (91)
1	Guava	3	.10	3	C	16	90 (84)
0.75	Guava	4	.01	1	D	104	59
	Guava	4[g]	.01	1	D	104	12
Aldrin—							
(25% WP) 2.2	Banana	3	.16	4	D	105	60
	Banana	3	.16	4	D	20	75
2	Guava	5	.33	6	D	5	86
1.75	Guava	6	.33	2	D	13	41 (61)
	Guava	3	.33	4	D	3	94
1.5	Banana	4	.25	4	C	14	57
	Banana	4	.25	4	D	14	47
	Banana	3	.20	2	C	128	44
0.8	Guava	3	.33	4	D	3	91
0.4	Guava	1	5.0	3	C	20	88
(25% Em) 2.2	Banana	3	.16	4	D	105	69
	Banana	3	.16	4	D	20	49
1	Guava	1	3.0	2	C	2	42
0.8	Guava	3	.33	4	D	3	62
(Xylene, oil) 45 grams	Banana	1	65.0	4	Fog	42	0
Chlordane (50% WP) 2.5	Banana	3	.20	2	C	128	0
DDT—							
(97% WP) 5	Banana	3	.16	4	D	105	88
	Banana	3	.16	4	D	20	94
(75% WP) 12	Guava	4	.01	2	D	2	91
4	Papaya	4	.05	3	C	26	96 (90)
	Guava	5	.33	6	D	5	95
	Guava	3	.10	3	C	14	62 (55)
2.25	Guava	3	.33	4	D	3	74
2.25+oil 2.25	Guava	3	.33	4	D	3	43
2.2	Guava	5	.01	3	C	14	50
(50% WP) 10	Guava	4	.05	3	D	6	98
6	Mango	2	.5	4	C	13	96
5.25	Banana	3	.16	4	D	105	82
	Banana	3	.16	4	D	20	98
5.25+oil 7	Banana	3	.16	4	D	20	93
5+EPN (25% WP) 0.5	Mango	2	.5	4	C	13	95
4	Guava	5	.33	6	D	5	94
3.5	Guava	6	.33	2	D	13	64 (65)
3.5+EPN (25% WP) 0.22	Guava	3	.16	4	D	20	92
3	Banana	4	.25	4	C	14	82
3+aldrin (25% WP) 0.3	Guava	6	.33	2	D	13	78 (78)
3+dieldrin (25% WP) 0.2	Banana	4	.25	4	C	14	69
3+EPN (25% WP) 0.4	Mango	3	3.0	3	C	4	92
2.5	Banana	3	.20	2	C	128	37
2.25	Guava	3	.33	4	D	3	76
2.25+parathion (25% WP) 0.14	Guava	3	.33	4	D	3	88

Insecticide Formulation and Pounds of Toxicant or Additive per Acre[a]	Host Sprayed	Number of Replicate Plots	Acres per Plot	Number of Sprays	Type of Spray[b]	Number of Larvae per Pound in Checks	Per Cent Mean Control[c]
1.5	Guava	2	4.0	3	C	16	90 (82)
(50% paste) 5.25	Banana	3	.16	4	D	105	64
	Banana	3	.16	4	D	20	85
3.5+EPN (25% WP) 0.22	Banana	3	.16	4	D	105	61
2.5	Banana	3	.20	2	C	128	51
(25% Em) 5.25	Banana	3	.16	4	D	105	70
	Banana	3	.16	4	D	20	86
2.5	Banana	3	.20	2	C	128	31
(Xylene, oil) 180 grams	Banana	1	35.0	4	fog	36	0
Dieldrin—							
(25% WP) 6	Guava	4	.01	2	D	2	75
3	Mango	2	.50	4	C	13	98
2.5	Guava	4	.05	3	D	5	98
2.2	Banana	3	.16	4	D	105	38
	Banana	3	.16	4	D	20	76
2	Guava	5	.33	6	D	5	97
1.75	Guava	6	.33	2	D	13	66 (64)
	Guava	3	.33	4	D	3	70
1.5	Papaya	4	.05	3	C	26	98 (97)
	Banana	4	.25	4	C	14	40
1.5+parathion (25% WP) 0.5	Guava	5	.33	6	D	5	96
1.25	Banana	3	.20	2	C	128	2
1	Papaya	5	.01	3	C	14	80
0.8	Guava	3	.33	4	D	3	86
0.75	Guava	3	4.5	3	C	9	95
0.5	Guava	2	4.0	3	C	19	90 (89)
0.4	Guava	1	5.0	3	C	18	84
0.25	Banana	4	.25	4	C	14	81
(25% Em) 2.2	Banana	3	.16	4	D	105	44
	Banana	3	.16	4	D	20	60
1.25	Banana	3	.20	2	C	128	0
Dilan—							
(50% WP) 5.25	Banana	3	.16	4	D	105	92
	Banana	3	.16	4	D	20	95
4	Guava	5	.33	6	D	5	90
3.4	Guava	3	.33	4	D	3	88
1.75	Guava	3	.33	4	D	3	85
(25% WP) 1.5	Papaya	4	.05	3	C	26	93 (87)
1	Papaya	5	.01	3	C	14	58
(80% Em) 4+malathion (50% Em) 0.75	Mango	3	3.0	3	C	4	93
(40% Em) 12	Guava	4	.01	2	D	2	62
(25% Em) 2	Mango	2	.5	4	C	13	92
1.75	Guava	3	.33	4	D	3	85
Heptachlor (25% WP)							
6	Guava	4	.01	2	D	2	68
1	Papaya	5	.01	3	C	14	52
Isodrin (25% WP) 2.5	Guava	4	.05	3	D	6	98
Lindane (25% WP)							
6	Guava	4	.01	2	D	2	94
1.25	Banana	3	.20	2	C	128	57
0.8	Guava	3	.33	4	D	3	84
Methoxychlor—							
(50% WP) 24	Guava	4	.01	2	D	2	75
20	Guava	4	.05	3	D	6	98
10	Papaya	4	.05	3	C	26	96 (95)
4	Papaya	4	.05	3	C	3	89 (87)
	Papaya	5	.01	3	C	14	51
2.5	Banana	3	.20	2	C	128	23
2	Papaya	5	.01	3	C	14	26
(25% Em) 2.5	Guava	6	.33	2	D	11	31 (37)
NPD (25% WP) 1	Guava	3	.10	3	C	14	0
Toxaphene (40% WP) 2.5	Banana	3	.20	2	C	128	39

[a] Identification of protein hydrolysates used:
PH, protein hydrolysate, enzymatic; PHYP, partially hydrolyzed yeast protein; LH, experimental enzymatic hydrolysate from lactalbumin; all Marvin R. Thompson, Inc.
YH, yeast hydrolysate, enzymatic; Nutritional Biochemicals Co.
SB, sauce base No. 2 (acid hydrolysate of corn protein); A. E. Staley Manufacturing Co.

[b] C refers to concentrated or semiconcentrated spray applied at 100 gallons or less and D to dilute spray at more than 100 gallons per acre.

[c] Unless otherwise indicated, average infestation reduction throughout 2-week period after each spray below that in check plots or in pre- and post-spray periods. Where sprays were applied at 3- or 4-week intervals, the reduction for the entire interspray period is given in parentheses.

[d] 1 week after sprays applied at weekly intervals.

[e] 66 days after application.

[f] 24 to 40 days after a single spray. Reduction for 20- and 10-pound demeton dosages at 40 to 75 days and 75 to 100 days were 69 and 55%, respectively.

[g] Applied to soil only.

1954. The protein hydrolysate-organic phosphorus bait sprays did not reduce the parasitization percentage. The tartar emetic sprays also appeared to be harmless to *oophilus*.

Nonbait Sprays.—In field tests with conventional sprays wettable powders were used almost exclusively because they had been found to be more effective than emulsifiable formulations as residual toxicants in the early phases of the investigations. Differences in the toxicity of topical applications found in the laboratory are unimportant under field conditions, since few flies present at the time of spraying at rates needed to insure effective residual action can survive, regardless of the formulation used.

EVALUATION OF DIFFERENT FORMULATIONS. The following evaluation of various formulations in sprays is based largely on data in part II of table 1, although use has also been made of other information obtained in more detailed laboratory and field studies.

Parathion.—This in a wettable powder formulation was the most effective insecticide used in both bait and nonbait sprays. At application rates of 2 or more pounds of toxicant per acre deposits on foliage had good residual action against adult flies for 2 weeks or more. Generally the residues killed all flies caged for 24 hours with treated foliage 1 to 4 days after spraying. In six of seven field experiments 2 to 6 pounds of parathion per acre gave 90 to 99% reductions in infestations for 2 weeks.

As reported by Steiner & Hinman (1952), vapors or air-borne particles from the deposits were effective for several days against flies not coming in contact with the sprayed surfaces, this effect being equaled only by dieldrin. In more recent tests parathion deposits on guava, during the first 24 hours after spraying at rates as low as 4 ounces per acre, killed 98 to 100% of the Mediterranean and oriental fruit flies in cages not in contact with the sprayed surfaces.

Parathion wettable powder proved to be excellent for use with methyl eugenol (Steiner 1952) in a poisoned bait for application to feeding stations to annihiliate oriental fruit fly males. Emulsifiable formulations, on the other hand, reduced the attractiveness of the methyl eugenol.

Less than half as much parathion as DDT was required for comparable results despite the shorter residual action of parathion. Since parathion has less differential toxicity to the three fruit flies present in Hawaii, it is more suitable for controlling mixed populations. In addition to the oriental fruit fly these may include the melon fly (*Dacus cucurbitae* Coq.) or the Mediterranean fruit fly (*Ceratitis capitata* (Wied.)). Parathion and some related compounds killed larvae hatching from eggs present in the fruit at the time of spraying, whereas most of the chlorinated hydrocarbons did not attain their maximum effect until a week or more after the first spray. Because of its quick action, parathion is especially useful on small acreages subject to a heavy immigration of gravid flies. At application rates needed for fruit fly control it was one of the least harmful insecticides to the parasite, *Opius oophilus*.

Since attempts to breed resistant strains of the oriental fruit fly through constant exposure to parathion have failed, it is highly improbable that long-continued field usage of parathion would lead to such development.

In preliminary field tests with mature larvae, parathion was an outstanding soil toxicant for as long as 30 days after application in sprays to the soil surface at 5 or more pounds per acre.

Parathion produced an extremely toxic aerosol fog. Wind-borne fogs gave complete kill of caged oriental fruit flies as far as $\frac{1}{8}$ to $\frac{1}{4}$ mile from the discharge point, when applied at the rate of $\frac{1}{4}$ ounce of parathion per acre. However, presently parathion is not considered safe for such usage.

Parathion is toxic to aphids, mealybugs, and green scales, all of which secrete honeydew, reported by Hagen (1953) to be a source of essential food elements for fruit flies. In several long-term small-plot tests parathion became more effective after three or four sprays, even though there was little accumulation of residues on the fruit and foliage. The minute quantities that are present, however, can quickly kill fruit flies that consume them. Further investigation is needed to determine whether the increased effectiveness of parathion after several sprays is a result of (1) more nearly complete or uniform contamination of natural sources of water and food, including honeydew, (2) evacuation of flies from the area in search of essential dietary elements should the local supply be destroyed, or (3) accumulation of residues on the soil or low vegetation to a point where flies attracted to fallen fruit or emerging from the soil succumb. In one experiment where a wettable-powder spray was applied once to guava at 24 pounds of parathion per acre, the mean control during the second and third months exceeded 70 and 60%, respectively.

The effectiveness of DDT also improves after several sprays have been applied, but this is doubtless because of the accumulation of deposits and better coverage.

In nonbait sprays parathion gave inadequate control when used at less than $1\frac{3}{4}$ pound per acre. In rainy periods its effectiveness declined more rapidly than that of DDT.

EPN.—This insecticide in wettable-powder sprays was compared with parathion in six experiments. At five dosages ranging from $1\frac{1}{2}$ to 6 pounds per acre it was inferior, but at $1\frac{1}{4}$ pound neither insecticide performed well.

EPN residues on guava and mango fruit were comparable to those left by parathion. The residual effectiveness of EPN on foliage was longer than that of parathion, but the toxicity of its vapor or air-borne particles was much less. In limited observations EPN was no more deleterious to parasites than parathion.

Demeton.—This systemic insecticide (in Systox) was tested only on guava. At 1 to 2 pounds per acre it gave 90 to 98% control for 2 weeks after each spray and 81 to 91% for 4 weeks. The toxicity of residues on foliage was negligible after 2 to 4 days of weathering. Fumigant action, which was less than half that of parathion on the day of spraying, completely disappeared within 4 days.

Demeton residues did not prevent oviposition, but in guavas the insecticide or substances derived from it were toxic enough to prevent larval survival or hatch for 8 or more weeks after a spray of 2 pounds of demeton per acre. Drenching sprays that contained 1 pound or less per 100 gallons were no more effective than similar amounts of parathion. Demeton showed no tendency to depress parasitism.

Malathion.—When used alone, this insecticide gave good control at 5 and 6 pounds per acre but not at the 1-

pound rate commonly used in bait sprays. Malathion is highly toxic to fruit flies when ingested, and weathered deposits that failed to kill flies confined with them for 24 hours could be made effective by the application of protein hydrolysate over them. Malathion's residual effectiveness in the absence of attractive nutrients rarely lasts more than 7 days when applied in full-coverage sprays at less than 3 pounds per 100 gallons.

DDT.—Of all materials this one was tested most extensively. It was included in nearly all experiments as a standard of comparison. At dosages below 4 pounds per acre in 50 or 75% wettable-powder sprays it often gave poor control, and it failed occasionally at 4 pounds or higher rates. When DDT was applied to guava at 2¼ to 4 pounds per acre, residues averaged 8.2 p.p.m. on the day of spraying and 4.8 to 6.9 p.p.m. after 24 hours. Residues on mangoes averaged 3.4 p.p.m. soon after the application of 6 pounds per acre. With any increase in tolerance of local fly populations to DDT, adequate control would not be possible at dosages that would avoid excessive residues on ripening fruit. Laboratory studies still in progress indicate that exposure to DDT residues can lead to the development of a moderately resistant oriental fruit fly strain in 15 generations.

Wettable-powder formulations of DDT were more effective than emulsifiable and paste types. The addition of oil (Orthol K emulsion) did not improve the performance of DDT wettable powder, but EPN or parathion supplemented DDT effectively. This suggests the possibility of improved control with a reduction in residue hazards, along with good control of *Paratetranychus insularis* McG. This mite caused considerable foliage injury in unsprayed mango plots as well as in those sprayed with Dilan or DDT. The substitution of aldrin or dieldrin for some of the DDT resulted in a spray mixture less effective than DDT alone.

DDT was ineffective as a soil toxicant for fly larvae or pupae. In an aerosol fog applied at 180 grams per acre, DDT killed most of the caged flies in a 35-acre banana plot, but it failed to reduce infestations because of the absence of residual action and the constant influx of flies.

DDT depressed parasitization of the oriental fruit fly more than parathion but much less than either dieldrin or aldrin. It had less effect on parasitism in infestations in guava than in banana, mango, or papaya. In such fruits 2 to 4 pounds per acre reduced parasite emergence 75%.

Methoxychlor.—This insecticide in a wettable-powder spray gave good results only at high dosages of 10 to 20 pounds per acre. The tests suggested that it may be useful in small-area control operations where cost is not an important factor and where more toxic insecticides would be unsafe. Foliage deposits had a long residual action. Parasite emergence was depressed significantly at application rates above 2½ pounds per acre.

Dilan.—This insecticide was erratic in its performance. In general, the wettable powders were most effective, although good control on mangoes was obtained with the emulsifiable formulations, alone or when combined with malathion. When applied at 4 pounds per acre to guavas, Dilan left residues of 10.8 p.p.m. on the day of spraying, 5.8 p.p.m. 6 days later, and 3.2 p.p.m. on the 12th day in an experiment where the infestation reduction averaged 90%. In most of the comparative tests Dilan depressed parasite emergence to the same degree as DDT.

Dieldrin.—This insecticide was slow in its action. Oriental fruit flies were able to crawl over fresh residues from sprays containing 2 pounds per 100 gallons for as long as 2 hours and deposit many eggs before showing any effects. However, its residual toxicity was unusually high, and residues on foliage were usually more effective than those of DDT for 3 to 4 weeks. The toxicity of vapors or air-borne particles of dieldrin was equal to if not greater than that of parathion, but here also the delay in toxic action was evident.

Dieldrin appears to be an outstanding fruit fly insecticide for large-scale use in foliage applications at ½ pound or more per acre. However, it is unsuited for small-area use if nearby infested areas are left untreated. There is evidence that ovipositing flies are either attracted into the sprayed area or after having contacted the poison are stimulated to deposit many eggs before succumbing to its slow but sure action. Increases in infestations of 50% or more were often encountered during the first few days after applications, especially in small banana and guava plots nearest outside sources of flies. When dieldrin sprays were fresh, best results were sometimes obtained with the lowest concentration used. For example, in one well-replicated series of tests on bananas, 1½ pounds per acre applied five times reduced infestations 32, 48, and 59% after 1, 2, and 3 weeks, respectively. Where only ¼ pound per acre was applied, the respective average reductions were 78, 83, and 25%. DDT at a 3-pound rate gave 92, 72, and 63% control during the same periods. In ¼-acre guava plots replicated three times 4/5 pound of dieldrin per acre gave 86% control after each of four sprays compared to only 70% where 1¾ pounds were applied.

Among several formulas applied to samples of ripe bananas that were subsequently exposed to fly attack for five days in five locations, ½ pound of parathion per 100 gallons effected a 99% reduction in an infestation of 30 larvae per pound in the controls. Dieldrin at ½ pound per 100 gallons gave only a 30% reduction, at 2½ pounds, 53%, and at ⅛ pound there was an increase of 33%. Aldrin and chlordane at low concentrations also caused a slight increase in infestation although less than dieldrin. In olfactometer tests dieldrin was significantly attractive to oriental fruit fly adults, but in field tests it was ineffective as a bait-trap lure.

As a soil toxicant dieldrin was outstanding. It remained highly effective against pupating larvae for several months at dosages as low as 5 pounds per acre. Residues from five sprays of dieldrin, each applied to guava foliage at 1½ pound per acre, gave as much as 68% control of larvae or pupae in the soil under the trees 78 days after the final spray. The effect of accumulating soil residues could not be demonstrated in small plots because the emerging flies had 8 or more days to redistribute themselves before reaching sexual maturity.

In aerosol fogs the toxicity of dieldrin to caged flies 500 feet from the generator outlet was exceeded only by parathion or Pyrolan.

Steiner & Hinman (1952) reported from 2 to 11% parasitization in dieldrin and aldrin plots compared with 26 to 69% in plots sprayed with other insecticides. These insecticides continued to depress parasite recover-

ies from infested fruit more than any others tested. How much of this resulted from adult parasite mortality was not determined. There was, in fact, a greater mortality among parasitized larvae or pupae than among unparasitized. Like parathion, dieldrin sprays killed some larvae inside the fruit. In recent laboratory tests with soil toxicants, Tamashiro & Sherman (1955) found evidence of a latent toxicity that may cause mortality of adult flies soon after emergence.

Though presently considered too dangerous for food crops, dieldrin may prove useful as a soil toxicant or in large-scale, low-dosage applications to control fruit flies on inedible hosts.

Aldrin.—This insecticide should be most useful as a soil treatment, being nearly as effective as dieldrin against fruit fly larvae and pupae. Like dieldrin, it is not dependable as a host spray except for large-scale use. The period of its residual activity is shorter than that of dieldrin, and its toxicity to parasites and its fumigating action are slightly less. In an aerosol fog applied at the rate of only 45 grams per acre to a 65-acre block of bananas, aldrin greatly depressed trap catches of flies for 24 hours, but it had no observable effect on the subsequent infestations.

Miscellaneous Materials.—Among the other insecticides tested, toxaphene and chlordane at 2½ pounds, heptachlor at 1 and 6 pounds, NPD at 1 pound, and Aramite at 2/5 pound per acre were of little value. Lindane was less effective than parathion, dieldrin, or aldrin in the killing effect of its vapors or air-borne particles. It also had little residual activity at economical application rates. Lindane depressed parasitism much less than dieldrin or aldrin. Metacide was an effective toxicant for use with the protein or yeast hydrolysates in bait sprays, but less effective than equal amounts of parathion. Isodrin at 2½ pounds per acre gave 98% control on guava, the same as that obtained with dieldrin.

Pyrolan has recently supplanted parathion as the preferred insecticide for use with methyl eugenol in feeding stations to annihilate male oriental fruit flies (Steiner & Lee 1955). An emulsifiable formulation applied to mangoes at the rate of 3 pounds of Pyrolan per acre gave the same control (93 to 95%) as parathion in comparable applications. On small guava plots 2 pounds per acre equaled 1 pound of parathion supplemented with protein hydrolysate and sugar (81 to 83%) control). The fumigating action of fresh deposits from 2 pounds of Pyrolan per acre was nearly equal to that from 1 pound of parathion, the respective mortalities being 87 and 98% during an 18-hour exposure period. Pyrolan was the most effective compound tested in aerosol fogs; as little as 7 grams per acre killed 98% of the flies that were caged 800 feet downwind. In one test complete mortality of both the oriental fruit fly and the melon fly was obtained 1300 feet from the discharge point. The discharge rate was 2 ounces per 100 feet traversed by the fog generator across an 8-m.p.h. wind.

Dilute vs. *Concentrate Sprays.*—The results presented in parts I and II of table 1 indicate that the method of application and the amount of water used in the sprays are unimportant provided coverage is reasonably uniform. In several comparative tests of aldrin, dieldrin, DDT, and parathion, each insecticide performed as well in concentrate as in dilute sprays. This permits the use of the application method best suited to the control of any other pests requiring attention when fruit fly sprays are needed.

CONTROL RECOMMENDATIONS.—On the basis of present knowledge, should an oriental fruit fly infestation occur on the mainland, the procedures most likely to insure eradication are immediate installation of poisoned methyl eugenol feeding stations throughout the infested zone as well as a buffer area to annihilate male flies and application of malathion- or parathion-protein hydrolysate bait spray to all hosts. With the phenomenal attractiveness of methyl eugenol to male oriental fruit flies, 40 or more well-distributed feeding stations per square mile, each impregnated with a mixture of this material and an insecticide such as Pyrolan, would destroy practically all emerging males before they could become sexually mature. Application of the bait sprays at 2- to 3-week intervals would soon eliminate gravid flies and those fertilized before the feeding stations are established, along with the occasional females that might develop fertile eggs thereafter because of failure of the methyl eugenol odor to reach all emerging males.

The most effective and practical bait spray appears to be one in which from 2 to 4 pounds of a 25% parathion or malathion wettable powder and 1 pound of an effective protein hydrolysate in 3 to 150 gallons of water is applied per acre. The quantity of insecticide per acre depends on foliage density, and the quantity of water on the type of spray equipment. Numerous experiments have shown that a coarse, well-distributed spray will give the best results. Application of bait spray to nearby ornamentals and vegetation may be desirable if the infestation is well established. There seems to be little justification or need for blanket applications in areas where methyl eugenol traps fail to demonstrate the presence of flies.

The treatment of soil under producing hosts may be a valuable supplemental eradication procedure where adequate coverage with poisoned feeding stations and the bait spray cannot be obtained. Dieldrin, aldrin, parathion, and lindane at 5 to 10 pounds per acre have been expecially effective against larvae and pupae in the soil.

For use on commercial fruit plantings under Hawaiian conditions, the malathion bait spray is the most practical because of the short interval permitted between application and picking. A recommendation for the use of this spray on mango, passion fruit, and guava has been approved (Steiner 1954). Parathion is even more effective and dependable than malathion for use in a protein hydrolysate bait spray, but the low tolerance of 1 p.p.m. for this material imposes limitations on its use.

SUMMARY.—Promising insecticides for control of the oriental fruit fly (*Dacus dorsalis* Hendel) were evaluated in 26 field experiments involving more than 180 tests on more than 600 plots from 1950 to 1955. The tests were conducted in Hawaii principally on mango, banana, guava, and papaya. Infestations in unsprayed guava check plots ranged from 2 to 104 larvae per pound of fruit and in unsprayed mangoes from 2 to 32 larvae per pound.

Best results were obtained with bait sprays containing parathion or malathion wettable powders and certain enzymatic or acid protein hydrolysates. Where parathion

bait sprays were applied at 2-week intervals, infestation reductions of 93 to 100% were obtained in 13 of 21 tests at rates of only ½ to 2 pounds of parathion per acre. Malathion bait sprays were only slightly less effective.

When applied in conventional residual-spray treatments, wettable-powder formulations of parathion, DDT, Dilan, and methoxychlor were moderately to highly effective at rates above 3 pounds of the toxicant per acre. Malathion gave good control at 5 to 6 pounds per acre. Dieldrin and aldrin, which sometimes proved highly effective at very low dosages on isolated or large plots, often failed at much higher dosages on small plots subject to reinfestation from nearby unsprayed areas. These two insecticides were the most harmful to oriental fruit fly parasites or to parasitized larvae of any tested. Each was promising as a soil toxicant, and residues from conventional foliage sprays after reaching the soil added substantially to the control by preventing fly emergence. Thorough applications of demeton sprays at 2 or more pounds per acre gave excellent control of infestations in guava, sometimes for extended periods principally because of the systemic action of this material. Pyrolan was the most effective toxicant tested in aerosol fogs and is preferred for use with methyl eugenol on bait stations for attracting and killing male flies.

On the basis of these and other experiments, the protein hydrolysate-parathion or -malathion bait sprays and methyl eugenol-pyrolan bait stations are recommended for use in any large-scale control or eradication campaign that might involve the oriental fruit fly.

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House Fly Control with Parathion and Diazinon Impregnated Cords in Dairy Barns and Dining Halls[1]

H. F. SCHOOF and JOHN W. KILPATRICK

The effective use of cords impregnated with organophosphorus compounds for control of house flies has been reported by Maier & Mathis (1955), Kilpatrick (1955) and Kilpatrick & Schoof (1956). In these studies, it was shown that cord, 3/32″ diameter, impregnated with 10% parathion provided season-long control of house flies in dairies, whereas the same cord immersed in 25% Diazinon solutions was effective for periods of only 6 to 8 weeks. In 1955, further studies were conducted to evaluate cord-bait applications, the effectiveness of 3/16″ diameter Diazinon-treated cord in dairy barns, and the comparative efficacy of parathion versus Diazinon cords inside military dining halls.

METHODS.—The parathion-treated cords (3/32″ diameter) were impregnated by immersion in a 10% parathion-xylene solution with a resultant dosage of approximately 100 mg. of parathion per linear foot of cord. Through chemical analysis it was found that by using a dipping machine uniform impregnation of cord could be obtained by complete submergence of the cords for a period of 2 minutes. Cotton cords 3/32″ and 3/16″ in diameter treated in a 25% Diazinon-xylene solution received an estimated dosage of 150 and 600 mg. of toxicant per linear foot, respectively. Cord installation was at the rate of 30 linear feet of cord per 100 square feet of floor area. In each treatment, cords were suspended vertically, either directly from the ceiling or from horizontal cords (treated) extending from wall to wall near the ceiling (figure 1). At the dairies, cords were installed in the milking barn, feed room, and all animal pens or shelters. At military food establishments cords were installed in the dining halls, kitchens, storerooms, and beneath the overhang at the entranceways.

The efficacy of the dairy treatments was appraised by

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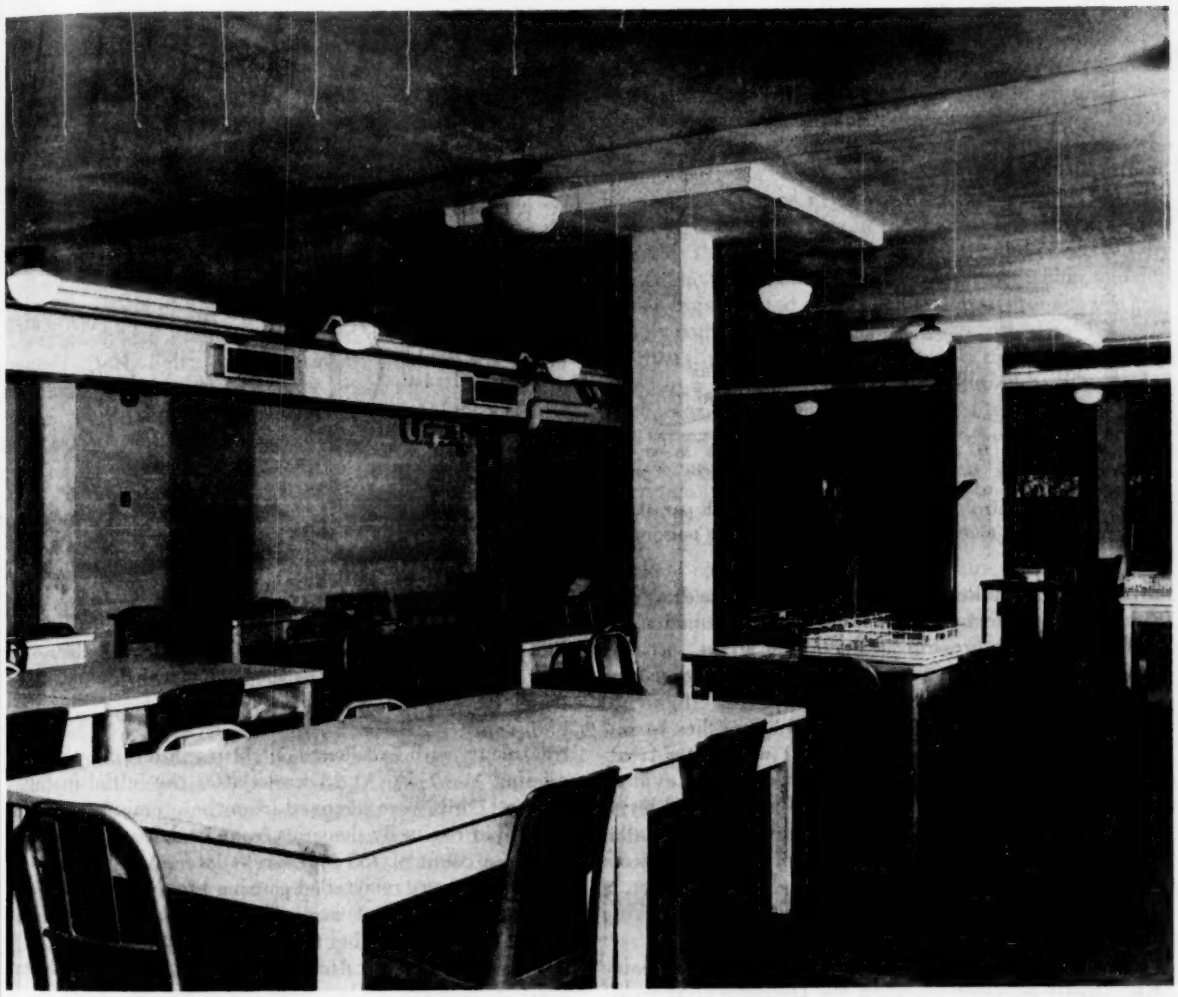

FIG. 1.—Typical installation of impregnated cord in a military dining hall.

making five fly grill counts (Scudder 1947) at each of the four general type locations (stanchions, barn floor, entrance, and feed room). The highest counts from each of the four locations were averaged to obtain a weekly fly index. In military dining halls the treatments were evaluated by obtaining total fly counts in the kitchen and dining hall. In each establishment, counts were made during the same time interval each week to avoid insofar as possible variation in the behavioristic pattern of fly activity (Kilpatrick & Quarterman 1952). Indices of 1 to 10 flies represented excellent control, of 10 to 20, satisfactory control.

Dairy (Ra) received treatment with parathion impregnated cord (3/32″ diameter) supplemented with seven treatments of a poison bait containing 0.1% DDVP and 10% sugar. Bait applications (2 gallons per treatment) were made on a 3- to 12-day basis over a 7-week period. A second dairy (T) was treated with parathion impregnated cords (3/32″ diameter) alone while in the third dairy (Kn) Diazinon cords (3/16″ diameter) were installed.

One military dining hall was treated with parathion-impregnated cords (3/32″ diameter) and two with Diazinon-treated cords (3/32″ diameter). One dining hall served as an untreated check.

RESULTS.—The results of the parathion cord treatments in dairies are shown in figure 2. House fly populations at Dairy "Ra" (cord plus bait treatment) and dairy "T" (cord treatment alone) were at relatively high levels prior to treatment. The installation of cords plus the use of DDVP poison baits immediately reduced fly populations at dairy "Ra" to control levels 1 day after treatment. Three bait treatments were applied during the first 8 days of post-cord installation with excellent fly control being achieved. Four additional bait treatments applied on a weekly basis reduced fly indices, but fluctuating populations at levels above control were evidenced. After cessation of the bait treatments, fly indices continued to decline and reached satisfactory fly control levels 1 week later. Except for 5 weeks when indices were between 20 and 22 flies per grill count, satisfactory control was obtained with the cords alone for the remainder of the fly season. The erratic fly indices at this dairy, along with the failure to achieve control throughout the season, may be attributed in part to the lack of normal sanitation practices. Excrement in calf pens was never re-

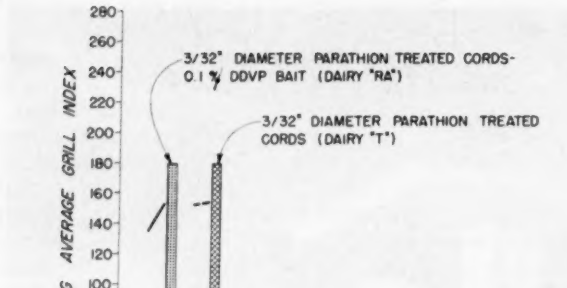

FIG. 2.—Fly control in dairy barns treated with parathion-impregnated cords alone and in combination with a poison bait

moved, and that taken from milking barn twice daily was stored within 25 feet of the milking barn, thus increasing the fly population pressure to maximum levels.

At dairy "T" with parathion cords alone a higher level of fly control was achieved than at dairy "Ra" (figure 2). At dairy "T" immediate reduction of house flies to satisfactory control levels was obtained 1 week after treatment and slowly reducing fly populations were evident throughout the entire fly season. Excellent control levels were reached 6 to 7 weeks after the initial cord installation. As with dairy "Ra," removal of excrement from calf pens was at a minimum at this dairy, however, a routine practice of spreading manure from the milking barn was followed.

The most spectacular fly control was obtained at dairy "Kn" (figure 3) which received the Diazinon cord treatment. Of all the dairies treated, dairy "Kn" has the largest, poorly sanitated calf pen area, however, satisfactory control levels were obtained within 2 weeks after

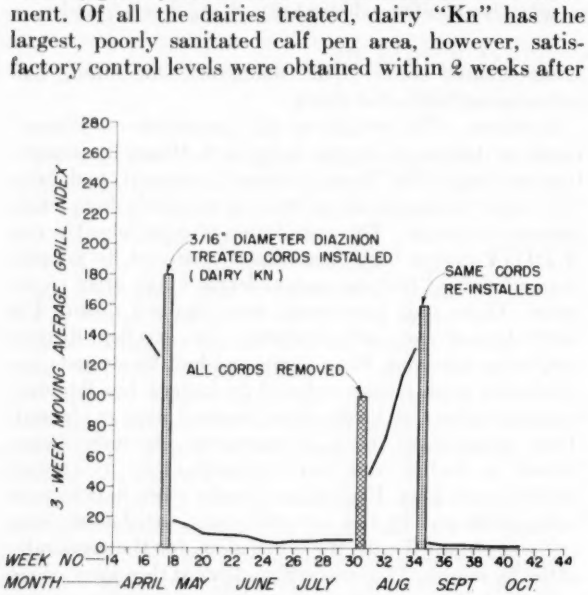

FIG. 3.—House fly control in a dairy barn treated with Diazinon-impregnated cords.

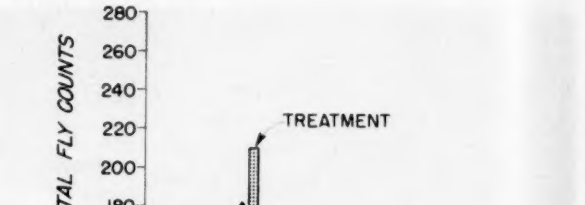

FIG. 4.—Total house fly counts in military dining halls treated with either parathion or Diazinon impregnated cords, 3/32" diameter.

treatment, with excellent control prevailing for the succeeding 11 weeks. At 13 weeks after the initial installation, all cords were removed from the premises, resulting in a rapid rise in fly densities from an average grill count of 6 to a count of 133 four weeks later. At this time the same cords were reinstalled causing the average fly count to drop to 4 within 1 week. Excellent control was maintained for the remaining 6 weeks of the fly season.

The results of parathion and Diazinon cord installations in military dining halls for house fly control are shown in figure 4. The extent of control with Diazinon impregnated cords parallels data obtained from tests in dairies (Kilpatrick & Schoof 1956) in which 6 to 8 weeks of excellent control were achieved. The two Diazinon treatments show a definite loss in effectiveness at and after the eighth week. Satisfactory fly control was maintained with parathion impregnated cords for the entire 13-week observation period. Total fly counts in the parathion treated dining hall ranged from a low of 6 to a high of 17 as compared with a low of 59 and a high of 182 in the untreated check.

DISCUSSION.—At dairy "Ra" approximately 1 month after the cord installation and after the sixth poison bait treatment, nighttime observations were made to ascertain a possible reason for the unsatisfactory fly indices present. Prior to the observations, the feed room floor was swept clean over an area of 450 square feet. Counts of flies resting on cords were then made on a 5 to 15-minute basis beginning at 6:25 P.M. At 6:45 P.M., up to 13 flies per 18" of cord were observed. At this time a total of 92 flies was counted on cords hanging from one rafter, while on the rafter itself, only two flies rested. At 7:00 P.M. counts of dead or knocked-down flies on the floor averaged approximately 75 flies per sq. yd., the flies being observed to fall almost continuously. By 8:30 P.M. the feed room was

almost devoid of live flies, attesting to the continued residual effectiveness of the cords. On the following morning there was a steady influx of flies from outdoor areas into the feed room. In the main, these flies were the specimens which were recorded on the grill inspection even though at the end of the day they were killed by alighting upon the treated cords. These observations emphasize the need for sanitation practices which will prevent the replenishment of fly populations at a rate higher than the mortality rate being achieved by chemical means.

Cotton cords of 3/16″ diameter impregnated in a 25% Diazinon-xylene solution provided the most effective control of any dairy cord treatment, residual activity of the cords still being effective 24 weeks after installation. These results were an improvement over those obtained at the same dairy in 1953 with parathion impregnated cords 3/32″ diameter, 10% solution (Kilpatrick & Schoof 1956). However, in 1954, 3/32″ diameter cords impregnated in a 25% Diazinon solution were effective for only 6 to 8 weeks in dairy barns. As the thicker cord (3/16″) absorbs a greater amount of Diazinon than the 3/32″ diameter cord, this factor presumably is the reason for its longer residual action.

On a comparative basis, 3/16″ diameter cords dipped in 25% Diazinon cost five times as much per unit length as 3/32″ diameter cords impregnated in a 10% parathion solution. However, on an over-all cost basis, including labor for installation, the expense of the complete cord treatment is only about twice that of parathion. Despite the cost differential, the advantage lies with the Diazinon treated cords because of the low mammalian toxicity of Diazinon compared with parathion. At the 25% concentration level used in cord preparation, Diazinon, based on known acute LD_{50} levels is 4 to 8 times less hazardous than parathion. This fact, plus the approval of Diazinon for use in dairy barns as a residual treatment, opens the way for operational use of the cord technique. Since the present data refer to only one dairy barn, further studies are necessary to determine if Diazinon-treated cords are as effective as parathion-treated cords on a general basis.

Chemical analysis of air samples (20 liters) taken in close proximity to freshly dipped and installed cord, have shown the concentration of parathion in the air to be not more than 0.02 micrograms/liter. This low level is considered insignificant from the standpoint of toxicity hazard. In addition, routine checks on the cholinesterase levels of workmen preparing and handling treated cords, have shown only slight variations in either erythrocyte or plasma levels. Also, no reports or observations of adverse effects upon the exposed personnel or animals have been noted either from the dairy or dining hall installations.

Despite the recognized toxicity of parathion, the widespread use of commercially-prepared parathion-impregnated gauze in Denmark (Wichmand 1953) for the control of house flies in rural areas has demonstrated that this technique may be used safely and effectively by the public. It thus appears that both parathion and Diazinon impregnated cords offer a definite promise as a general fly control measure.

SUMMARY.—Cotton cords (3/32″ diameter) impregnated in a 10% parathion-xylene solution have provided satisfactory to excellent house fly control in dairy barns and in a military dining hall near Savannah, Georgia. Cotton cords of 3/32″ diameter impregnated in a 25% Diazinon-xylene solution provided 6 to 8 weeks of excellent to satisfactory house fly control in a military dining hall, while similarly treated cords, 3/16″ diameter, gave excellent control in a dairy for an entire season from May through the middle of October. Removal of the Diazinon-treated cord from the dairy in August caused a rise in fly densities from 6 to 133 flies per grill count in 4 weeks. Subsequent reinstallation of the same cords reduced the indices to below four flies per grill count for the balance of the season.

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Reduced Dosages of Insecticides for Corn Rootworm Control[1]

J. W. Apple,[2] *University of Wisconsin, Madison*

During the past 8 years there has been a steady increase in the use of chemicals for the control of the northern corn rootworm (*Diabrotica longicornis* (Say) and the western corn rootworm (*D. virgifera* Lec.) in the north-central states. Research work for the most part has centered around the use of broadcast application of chemicals and as a consequence, this method of control has been used most extensively by midwestern farmers. One pound per acre of aldrin or heptachlor is most frequently recommended and used for broadcast treatments. More recently, there has been an interest in effecting rootworm control by the incorporation of an insecticide in starter-fertilizer, and in this case, one-half pound or more of an insecticide is used on each acre. The purpose of the present study was to evaluate reduced dosages of aldrin and heptachlor in both methods of application in an attempt to establish the validity of the 1- and the ½-pound per acre rates currently being used.

Workers in Nebraska were the first in the Corn Belt to advocate the use of insecticides for the control of rootworms on corn. Hill *et al.* (1948) obtained 72.8 to 96.4% reduction of rootworms (mostly western species) by the use of 0.5 to 2.0 pounds of g-BHC per acre as pre-plowing broadcast sprays. Severe plant lodging was eliminated (19.1% severe lodging in control) by g-BHC but yields were not significantly different from the control. DDT at 5 and 10 pounds per acre was only moderately effective in protecting plants from rootworms.

Cox & Lilly (1953) used insecticides in starter-fertilizer during 1951 and obtained excellent plant protection from the northern corn rootworm. Rootworms were reduced from 17.9 per hill in the control to 4.1 or less by the use of 1 pound per acre of aldrin, dieldrin or chlordane mixed as wettable powders into fertilizer. Severe lodging was reduced from 24.8% to 1.8% or less, and yields were increased from 28.0 bushesl per acre in the controls to an average of 36.0 bushels per acre by the use of the insecticides in fertilizer. These authors reported the results of two broadcast spray experiments where high rootworm control was obtained with relatively low dosages of several insecticides. In the first of these, 0.75 pound of g-BHC, dieldrin, chlordane and aldrin gave rootworm reductions of 97, 91, 78 and 75%, respectively. Gamma isomer of BHC and aldrin were used in the second test at a dosage of 0.5 pound per acre and rootworm control amounted to 88 and 84%, respectively, while the chemicals used at 1 pound per acre gave 95 and 93% control, respectively. In spite of the good rootworm control in both experiments, yields were not significantly different from untreated plots although severe lodging was eliminated.

Lilly (1954) used 0.5 pound heptachlor, 0.6 pound aldrin and 0.8 pound of chlordane per acre in starter-fertilizer in a 1953 test and obtained 68%, or better, rootworm control. No lodging occurred in any of the plots even though untreated rows had 19.8 worms per hill. Insecticide-treated rows failed to produce yields significantly different from the control, but among the three insecticides, chlordane-treated plots produced a significantly poorer yield than plots treated with the other two materials. In an evaluation of broadcast sprays applied before spring-plowing, 1 pound per acre of heptachlor, gamma isomer of BHC, endrin and aldrin gave worm reductions of 96, 95, 93 and 75%, respectively. Lodging was reduced from 79% in the control to 4% or less for the treatments and yields were increased from 49.2 bushels per acre in the control to an average of 100.5 bushels. Untreated plots in this test had 154 rootworms per hill.

Working on the western corn rootworm, Burkhardt (1954) conducted an extensive test with reduced dosages of four chemicals incorporated in starter-fertilizer. A quarter of a pound per acre of lindane, heptachlor and aldrin gave 90, 90 and 69% reduction in rootworms, respectively, while a ½-pound dosage of each chemical gave reductions of 100, 90, and 56%, respectively. Lodging was reduced from 47% in the control to 9% or less by the treatments. Chlordane at 0.5 and 1 pound per acre gave 93 and 100% control of rootworms, respectively, and protection from lodging comparable to the other six treatments. Yields were not significantly different in this experiment. In another test with insecticides in starter-fertilizer, Burkhardt used 0.5 pound of heptachlor and aldrin, and 0.75 pound chlordane per acre and affected rootworm controls of 99, 97 and 82%, respectively. Untreated plots showed 29% of the plants lodged while this adverse plant response was essentially eliminated with the three treatments. Yields were extremely low in all plots, part of this being attributed to adult feeding on the silks.

Methods and Materials.—Two methods of insecticidal application were employed for the evaluation of reduced dosages against the northern corn rootworm. Broadcast treatments were made to small replicated plots while starter-fertilizers containing insecticides were applied to entire rows with conventional four-row planters.

A 1954 evaluation of insecticides applied broadcast in a fifth-year corn field involved five toxicants and a limited comparison of two formulations; emulsion spray and attapulgite granules (30/60 mesh—AA grade). Emulsion sprays were applied to individual plots measuring 16×40 feet with a compressed-air (CO_2) sprayer while the one treatment with granules involved hand-distribution of previously weighed amounts. Unused spray material was measured to provide a basis for computing the actual quantities applied. Treatments were replicated four times in a randomized-block field design. Plowed soil was treated and plans called for the insecticides to be disked under the same day. Because of a misunderstanding, the farmer did not work the field until 4 days later thus exposing the chemicals on the surface of the soil to sunlight (78° F. max. air temp.) and wind but no rainfall.

[1] Approved for publication by the Director of the Wisconsin Agricultural Experiment Station. Accepted for publication June 14, 1956.

[2] The author wishes to acknowledge the assistance of E. M. Raffensperger in securing certain of the harvest data.

A comparison of insecticides applied broadcast in a 1955 experiment involved the use of aldrin, heptachlor, and endrin in a third-year corn field. Emulsion sprays were applied with a compressed-air sprayer to plots measuring 14 by 50 feet. Two granulated formulations were applied in this test with a typical lawn-seeder. Four replicates were employed with a 15-foot untreated buffer strip between each replicate, so as to minimize the movement of chemicals from one block into the plots of the next tier. All materials were applied to old corn stubble and within 2 hours after treatment the field was plowed in preparation for planting 10 days later.

Two experiments with insecticides in starter-fertilizer were conducted during 1955 in southern Wisconsin. Both tests were on fourth-year corn. Each farmer had purchased starter-fertilizer containing 10 pounds of aldrin per ton and these mixtures were included in the tests but in addition, untreated fertilizer of the same grades (4–16–16 and 5–20–20) was made available for non-insecticide treatments and for the incorporation of other insecticides and different concentrations of aldrin. Insecticides on 30/60 mesh AA grade attapulgite (20% aldrin, 25% heptachlor and 25% chlordane) and on No. 4 vermiculite (10% aldrin and 10% heptachlor) were blended into the fertilizer at each of the farms with the aid of a small concrete mixer. These "homemade" formulations resulted in less fertilizer being applied to the acre (up to 2.5%) than was the case with untreated fertilizer or where the toxicant had been incorporated in a proprietary mixture.

Seven insecticide variables and a fertilizer control were used at each of the farms. Four-row planters were employed thus making it possible to use a different treatment in each of the four fertilizer boxes. This procedure necessitated having four treatments in one block of rows while the remaining treatments appeared in an adjoining block. Each treatment appeared in four rows on the Florin farm and in 12 rows at the Wilson farm.

Data on surviving rootworms were not gathered from the various plots because of insufficient labor but it was established that worms were present and damage to corn roots ranged from moderate to heavy. Differences between treatments were based on the extent of plant lodging and yield samples. In the one test in 1954, a plant was considered lodged if it inclined more than 30 degrees from perpendicular while in 1955 a 45-degree inclination was used. These plant observations and yield samples were taken in 33.3 feet of the middle two rows of the four-row broadcast application plots, and in 33.3 feet of four rows and in 66.7 feet of five rows in the Florin and Wilson starter-fertilizer experiments, respectively. Field harvest weights were converted to bushels of No. 2 corn per acre after being corrected for differences in ear moisture at harvest time.

RESULTS.—The 1954 evaluation of insecticides applied as broadcast treatments showed heptachlor more effective than aldrin in protecting corn from rootworms under somewhat abnormal conditions (table 1). In spite of the fact that 0.55 pound of heptachlor per acre lay exposed on the surface of the soil during 4 days, this treatment resulted in good protection from early lodging and a 23.5% increase in yield, while aldrin at 0.46 pound per acre was little or no better than the control. When aldrin and heptachlor were used at dosages around 1 pound per acre, the commonly recommended rate, there was no real difference between the two chemicals. Chlordane at 1.67 pounds, BHC at 0.3 pound of gamma isomer and endrin at 0.5 pound per acre were approximately equal in effectiveness with the 0.55-pound dosage of heptachlor and the 0.85-pound rate of aldrin. The excellent performance of gamma isomer of BHC corroborates the work of Hill *et al.* (1948), but this chemical is not recommended for rootworm control in Wisconsin because of its tendency to remain in the soil and produce off-flavors in subsequent crops. There was no evidence that aldrin on granules was different in toxicity from aldrin applied as an emulsion spray. All plots in this experiment showed more plant lodging at harvest time than on July 30, but differences between treatments were approximately the same.

Broadcast application of insecticides immediately before spring plowing in the 1955 test (table 2), resulted in a marked reduction in lodging and as much as 18% increase in yield. At dosages near 0.5 pound per acre, heptachlor and aldrin brought about yield increases of

Table 1.—Evaluation of broadcast insecticides for the suppression of corn rootworm damage. 1954.

Insecticide (Pounds per Acre)	Lodging 7/30 (>30°) Per Cent	Angle (N+0.25)	Per Cent Reduction	Yield Bu./Acre	Per Cent Deviation
Aldrin					
0.25	50.1	45.0	13.2	81.7	−8.7
0.46	24.0	28.2 [a]	58.4	89.5	0.0
0.85	6.5	14.0	88.7	102.5 [a]	14.5
1.0 (gran.)	3.6	9.7	93.8	107.7	20.3
1.44	3.5	9.4	93.9	100.7	12.5
Heptachlor					
0.55	0	2.9	100	110.5	23.5
1.2	0	2.9	100	105.5	17.9
Chlordane					
1.67	0	2.9	100	102.2	14.2
BHC (g.i.)					
0.3	1.0	5.1	98.3	107.2	19.8
Endrin					
0.5	3.4	9.3	94.1	97.7	9.2
Untreated	57.7	49.9		89.5	
L.S.D. at 5% level		13.7		15.7	

[a] Numbers associated with particular vertical line are not significantly different (Multiple Range Test—Duncan, 1955).

Table 2.—Evaluation of preplowing application of insecticides for the suppression of corn rootworm damage. 1955.

Insecticide (Pounds per Acre)	Lodging at Harvest (>45°) Per Cent	Angle	Per Cent Reduction	Yield Bu./Acre	Per Cent Increase
Aldrin					
0.55	10.2	18.6	53.6	86.5 [a]	6.9
1.1	3.6	10.9 [a]	83.6	87.3	7.9
Endrin					
0.75 (gran.)	7.8	16.2	64.5	86.0	6.3
Heptachlor					
0.53	5.9	14.1	73.2	86.8	7.3
1.1	1.4	6.6	93.6	90.8	12.2
1.5 (gran.)	7.8	16.2	64.5	95.6	18.2
Untreated	22.0	27.9		80.9	
L.S.D. at 5% level		8.7		7.8	

[a] Numbers associated with particular vertical line are not significantly different.

7.3 and 6.9%, respectively, but such gains were not great enough to be significant. The reduced dosage of heptachlor resulted in 73.2% reduction of lodged plants which was not significantly different from the best treatment (93.6% reduction in plant lodging with 1.1 pounds of heptachlor per acre). Aldrin at 0.55 pound per acre reduced lodging 53.6% and this response was significantly poorer than the best treatment. As in the former experiment, there was no real difference between aldrin and heptachlor at dosages around 1 pound per acre. Heptachlor on clay granules did not show any advantage over an emulsion spray even though the former treatment involved 0.4 pound more toxicant to the acre. Endrin granules used at a rate that provided 0.75 pound of insecticide per acre failed to show particular promise for rootworm control.

Insecticides in starter-fertilizer at the Florin farm (table 3), produced significant improvements in yield and reductions in lodging. Based on lodged plants, there was no difference between heptachlor and aldrin when each was used at 0.25 and 0.5 pound per acre. However, yield comparisons revealed that 0.25 pound per acre of heptachlor gave essentially the same protection as a ½-pound rate of heptachlor while a quarter of a pound of aldrin was significantly poorer. A reduced dosage of heptachlor on vermiculite and on attapulgite clay gave comparable results when measured by plant lodging, but in terms of yield the insecticide on micaceous carrier did not compare favorably with the best treatment as did the ¼-pound rate of heptachlor on clay. The proprietary mixture of aldrin in fertilizer gave results commensurate with the dosage involved. Chlordane at 1 pound per acre afforded approximately the same protection as one-half of a pound of aldrin or heptachlor.

Table 3.—Corn lodging and yield following use of insecticides in starter-fertilizer for corn rootworm control. 1955.

Insecticide (Pounds per Acre)	Lodging at Harvest (>45°) Per Cent	Angle	Per Cent Reduction	Yield Bu./Acre	Per Cent Increase
Florin farm					
Aldrin					
0.25	9.9	17.4[a]	67.0	93.0	30.4
0.50	11.8	20.1	60.7	104.5[a]	46.6
1.00[b]	8.6	17.0	71.3	107.0	50.1
Chlordane					
1.00	7.9	16.3	73.7	106.6	49.5
Heptachlor					
0.25	10.6	19.0	64.7	104.0	45.9
0.50	3.3	10.5	89.0	110.5	55.0
0.25[c]	11.9	20.2	60.3	89.0	24.8
Fertilizer alone	30.0	33.2		71.3	
L.S.D. at 5% level		9.1		14.3	
Wilson farm					
Heptachlor					
0.22[c]	9.8	18.2	75.8	82.5	5.1
0.22	3.1	10.1[a]	92.3	88.2	12.4
0.44	2.6	9.4	93.6	89.0	13.4
Aldrin					
0.22[c]	7.6	16.0	81.2	82.2	4.7
0.22	5.1	13.0	87.4	85.2	8.5
0.44	7.9	16.3	80.5	86.7	10.4
0.87[b]	11.6	19.9	71.4	79.0	0.6
Fertilizer alone	40.5	39.2		78.5	
L.S.D. at 5% level		7.3		n.s.	

[a] Numbers associated with particular vertical line are not significantly different.
[b] Commercially prepared mixture.
[c] Insecticide on granulated vermiculite, other treatments involved granulated attapulgite.

The starter-fertilizer experiment on the Wilson farm (table 3) demonstrated the value of banded application of insecticide as a method for reducing plant lodging but no real differences in yield resulted. In this test, a dosage of 0.22 pound per acre of aldrin or heptachlor on an attapulgite carrier afforded approximately the same protection in terms of plant lodging as 0.44 pound of either insecticide. A reduced dosage of each chemical on a vermiculite carrier showed aldrin comparable to the best treatment but heptachlor was significantly less effective. For some unexplained reason, the commercially prepared aldrin-fertilizer mixture failed to provide the protection that would be expected from the dosage employed (0.87 lb./acre).

Conclusions.—Three experiments showed heptachlor more effective than aldrin in protecting corn plants from rootworms. Heptachlor was no better than aldrin when each was used at dosages around 1 pound per acre for broadcast treatments and around 0.5 pound per acre in starter-fertilizer. However, when application rates were reduced one-half, heptachlor continued to provide the protection afforded by the recommended rates while reduced dosages of aldrin were less effective than the recommended rates in four of the eight lodged plant and yield comparisons.

Summary.—Tests were conducted in 1954 and 1955 to evaluate reduced dosages of aldrin and heptachlor when used to protect corn from attack by the northern corn rootworm. The two insecticides were equally effective in reducing plant lodging and increasing yields when used at the recommended rate of 1 pound per acre in broadcast treatments and 0.5 pound per acre in starter-fertilizer but heptachlor produced better results than aldrin when dosage rates in each method of application were reduced one-half.

As broadcast treatments, chlordane (1.67 pounds/acre), gamma isomer of BHC (0.3 pound/acre) and endrin (0.5 and 0.75 pound per acre) proved to be equal to the 1-pound rate of aldrin and heptachlor. Chlordane at 1 pound per acre in starter-fertilizer was found to be as effective as 0.5 pound of aldrin or heptachlor.

Insecticides on attapulgite granules were no different in effectiveness from insecticides applied as emulsion sprays in broadcast treatments. In starter-fertilizer experiments, there was evidence that heptachlor on vermiculite granules was less effective than when present on an attapulgite carrier. This was not true with aldrin on vermiculite.

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Control of Early-Season Cotton Insects with Systemic Insecticides Employed as Seed Treatments[1]

C. R. Parencia, Jr., J. W. Davis, and C. B. Cowan, Jr.,[2] *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*[3]

Research with various methods of applying systemic compounds for cotton insect control has been in progress for several years. Workers at College Station, Texas concluded that the seed treatment method is superior for controlling both sucking and chewing species. They found that compounds that are most effective against chewing insects, such as the boll weevil, do not appear to be translocated when applied as sprays in the conventional manner, but some of them are translocated in sufficient quantity to kill many chewing insects when applied to the soil or to the seed (Ivy 1953). Much lower dosages are required for seed treatments than for soil application (Ivy *et al.* 1950). An activated-carbon carrier used for the seed coating permits much higher dosages of the systemic compounds to be tolerated by the seed and seedling plant than if a liquid dip is used, and the treatments are more persistent (Ivy 1952). The effectiveness of several compounds applied by this method against various species was reported by Ivy *et al.* (1954).

In Texas the control of early-season cotton insects is recommended by the Extension Service and is widely used by cotton growers in areas where thrips, cotton aphids, cotton fleahoppers, and overwintered boll weevils cause damage every year. Insecticides are applied as dusts or sprays. An effective seed treatment would have several advantages over conventional methods. After obtaining a stand from treated seed, the cotton grower would not have to be concerned about insects until midseason. The timing of insecticide applications, which is so important in the conventional early-season control program, would be of no consequence, since none would be needed. Tractors and manpower would be released for use in other farm activities. With seed treatment, grower participation in the early-season program would likely be greater than with the conventional method and would result in more effective early-season control.

Because of the need for the development of this method of early-season insect control and the favorable results obtained with it in laboratory tests, Am. Cyanamid 12008 and 12009 which were available in small quantities were tested as seed treatments near Waco in 1954. Encouraging results in this test and the availability of an apparently more effective compound, Am. Cyanamid 3911, made possible an expanded research program with this method of early-season insect control under field conditions in 1955. This paper reports the results obtained in the experiments against various early-season pests.

The compounds were obtained from the manufacturer as 50% activated-charcoal dusts. Planting seed was treated in a mechanically rotated drum about one month before planting. In 1954 fuzzy cotton seed was treated at the rate of 8 pounds of the dust per 100 pounds of seed. In 1955 mechanically delinted (reginned) seed was used. The seed that was to carry 1 pound of toxicant per acre was treated at the rate of 6 pounds of the mixture per 100 pounds when the planting rate was to be 33 pounds per care, and at 8 pounds of the mixture when a 25-pound planting rate was to be used. Four-row planting equipment available on the various farms was used.

In the experiment conducted in 1954, plot sizes for the Am. Cyanamid 12009 and 12008 treatments were 1.34 and 1.75 acres, respectively. An untreated check plot of about 4 acres was adjacent to the 12008 plot. The seed was planted in sandy soil on April 23. Planting rates were less than desired, 12008 at 0.57 pound and 12009 at 0.56 pound instead of the intended 1 pound per acre, and this probably reduced their effectiveness. Plants emerged to a stand on May 1. Seedling counts indicated that emergence was reduced 13 and 39% by the respective treatments.

Both treatments gave protection from thrips (*Frankliniella* sp.) infestations for 3½ weeks after plant emergence and some protection for an additional week. Am. Cyanamid 12008 gave slightly better control than 12009. Both treatments controlled the cotton aphid (*Aphis gossypii* Glov.) for 4½ weeks; after that aphid populations were eliminated through natural control in the check.

During the latter part of May and the first half of June plants in the seed-treatments plots were considerably taller than those in the untreated check. Plant appearance was normal in the treated plots, whereas plants in the untreated check were stunted and showed typical severe thrips and aphid injury for several weeks.

During the time the seed treatments were effective against thrips and the cotton aphid, infestations of the cotton fleahopper (*Psallus seriatus* (Reut.)) were too light for effectiveness against this insect to be determined. In late May and early June there was no appreciable difference in populations of overwintered boll weevils (*Anthonomus grandis* Boh.) between the treated plots and check. However, some boll weevils may have been killed by the seed treatments, since more of them would be expected to infest plants that were especially attractive as a result of early thrips and aphid control.

Squares began to form on a few plants in all plots on June 1. By that time thrips in the treated plots had increased so much that fruiting was delayed even though earlier protection had been afforded. Counts of fruiting forms (squares and blooms) in the latter part of June and July showed an appreciable increase in treated over the untreated area.

A small-plot and three large-plot experiments were conducted in 1955. In small-plot experiment 1 the plots were 0.1 acre in size and 12 rows wide, and they were arranged in randomized blocks with four replications of each treatment. In the large-plot experiments they were

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[3] In cooperation with the Texas Agricultural Experiment Station.

5 to 10 acres in size. In experiments 2 and 3 there were no replications of treatments but there were four replications in different fields in experiment 4.

In most of the experiments the seed was planted under good germinating conditions between April 18 and 25, and there were no differences in stands obtained from treated and untreated seed. In experiment 3 the seed was planted on April 8, and emergence was reduced by the 1 pound dosage of Am. Cyanamid 3911 when a heavy rain occurred on the day after planting. A fair stand was obtained from untreated seed but a poor stand from treated seed.

Cotyledons showed phytotoxic effects from the seed treatments in each experiment. On a plant percentage basis the injury was greatest from 1 pound of 3911 and least from 1 pound of 12008. There was no evidence of phytotoxicity to true leaves.

Besides the seed treatments, a toxaphene foliage spray treatment was included in experiment 1. Four weekly applications at 0.75 pound and two at 1.5 pounds of toxaphene were made between May 6 and June 11. A similar treatment was included in experiment 4, three applications at 0.75 pound and two at 1.5 pounds of toxaphene per acre being made between May 2 and June 13. The application that should have been made during the third week was omitted because of wet fields. A standard early-season control spray treatment was also included. One application of toxaphene at 0.75 pound and two at 1.5 pounds per acre were made at weekly intervals between May 24 and June 13. An application should have been made a week earlier, but fields were too wet for spray equipment to be used.

RESULTS.—*Leaf Miner.*—Dry weather prevailed for almost a month after the seeds were planted. Conditions were favorable for development of the serpentine leaf miner (*Liriomyza pusilla* (Meig.)) and cotyledons of a high percentage of seedlings were infested in all fields in the area. In experiment 1 infestations did not occur in the plots treated with the two dosages of 3911, but 6.3% of the plants in the 12008 plots and 53.8% in the check were infested. In large-plot experiment 2 located in the same field there was no infestation in the 3911 and 12008 plots while 72% of the plants were infested in the check. In the various replicates of experiment 4 an infestation was found in the 3911 plots in only one location, 6% of the plants being infested as compared with 48 in the check. After rains occurred there was no further development of infestations and none occurred on true leaves of treated or untreated plants.

Thrips.—In experiment 1 each seed treatment reduced the thrips infestation significantly below that of the check for 4 weeks after plant emergence (table 1). After 2 weeks the 1 pound dosage of Am. Cyanamid 3911 gave significantly better control than 12008 and toxaphene but only one application of toxaphene spray had been made at that time. After 5 weeks there were no significant differences among the seed treatments and the check but toxaphene gave significantly better control than each of the other treatments except 1 pound 3911. When all records were considered, each treatment reduced the infestation significantly below the check with no significant difference among treatments. The better control obtained with 1 pound of 3911 was reflected by better plant appearance in comparison with those of the other seed treatments. Plants in the untreated checks showed considerable thrips injury, although growth was less affected than when larger untreated areas were used.

Table 1.—Effectiveness of Am. Cyanamid 3911 and 12008 as cotton seed treatments against thrips in field experiments, Waco, Tex., 1955.

Treatments	Pounds of Toxicant per Acre	Average Number of Thrips per Plant on Specified Weeks After (Plant Emergence)						
		1	2	3	4	5	6	Average
Experiment 1								
3911	0.5	0.06	0.5	2.0	3.0	5.4	3.3	2.4
	1.0	.01	.3	1.5	1.9	4.6	3.6	2.0
12008	1.0	.06	.9	2.0	3.1	5.4	2.7	2.3
Toxaphene spray	1.0[a]	2.08	1.0	2.1	1.5	2.4	2.2	1.9
Untreated		1.85	2.4	4.7	9.4	5.8	4.2	4.7
L.S.D. at 5% level		.32	.5	1.2	2.4	2.3	1.0	0.5
Experiment 2								
3911	1.0	0	0.3	0.9	1.2	3.6	4.1	1.7
12008	1.0	0	1.4	2.6	3.2	4.3	5.2	2.8
Untreated		0.9	4.6	6.6	10.9	8.4	4.4	6.0
Experiment 3								
3911	1.0	0	0	1.3	1.6	3.2	1.2	1.2
Untreated		0.4	1.4	1.7	3.2	6.8	2.7	2.7
Experiment 4								
3911	1.0	0	0.2	1.9	1.9	2.8	5.0	2.0
Toxaphene spray	1.05[b]	0.6	2.1	2.0	4.2	2.7	1.0	2.1
Standard toxaphene spray	1.25[c]	.7	5.5	5.7	5.8	3.6	1.3	3.8
Untreated		1.4	6.2	5.7	9.7	8.6	4.6	6.0

[a] Average of 6 weekly applications after plant emergence.
[b] Average of 5 weekly applications after plant emergence.
[c] Average of 3 weekly applications beginning 3 weeks after plant emergence.

In the large-plot experiments thrips control with the seed treatments was obtained for 5 weeks and populations did not approach the checks until 6 weeks after plant emergence. Plants in untreated checks were recovering from thrips injury at that time.

Overwintered Boll Weevils.—In addition to the usual boll weevil records made in the various experiments, plants were caged at various intervals after plant emergence in the 1 pound 3911 plot and check in experiment 4. Overwintered weevils removed from hibernation cages were installed in the cages and control was determined by Abbott's formula. Results showed a progressive loss in effectiveness with plant growth. When weevils were introduced on caged plants 15 and 21 days after emergence, the control percentages after 3 days were 88 and 63.2, respectively. When they were introduced 28 days after plant emergence, control after 7 days was 33.2%. No kill was obtained on plants caged 32 or more days after emergence.

In experiment 1 (table 2) weevils were found in the untreated check and in the plots planted with seed treated with ½ pound of 3911 three weeks after plant emergence, but none were found in the plot planted with seed treated with 1 pound of 3911 or 12008. After 4 weeks they were found in all treated plots. Previous experience in overwintered boll weevil control in small-plot tests indicated that results could not be expected to be too reliable but certain conclusions are in order. The lower population in the check was due to the plants being less attractive because of thrips injury. Since populations were quite high in the plots planted with seed treated with ½ pound of 3911 after 3, 4 and 5 weeks, it is concluded that no

Table 2.—Effectiveness of Am. Cyanamid 3911 and 12008 as seed treatments against overwintered boll weevils in field experiments, Waco, Tex., 1955.

Treatments	Pounds of Toxicant per Acre	Boll Weevil Population per Acre on Specified Weeks After (Plant Emergence)					
		2	3	4	5	6	Average
		Experiment 1					
3911	0.5	—	69	275	172	34	138
	1.0	—	0	103	103	0	52
12008	1.0	—	0	138	0	69	52
Toxaphene spray	1.0[a]	—	0	34	241	69	86
Untreated		—	34	0	69	0	26
L.S.D. at 5% level		—	—	—	—	—	65
		Experiment 2					
3911	1.0	—	—	103	103	172	126
12008	1.0	—	—	69	69	138	92
Untreated		—	—	0	69	34	34
		Experiment 3					
3911	1.0	0	0	0	138	309	89
Untreated		138	69	138	206	34	117
		Experiment 4					
3911	1.0	17	17	164	69	103	74
Toxaphene spray	1.05[b]	0	0	60	34	17	22
Standard toxaphene spray	1.25[c]	0	34	129	26	26	43
Untreated		17	52	43	11	46	34

[a] Average of 6 weekly applications after plant emergence.
[b] Average of 5 weekly applications after plant emergence.
[c] Average of 3 weekly applications beginning 3 weeks after plant emergence.

appreciable control was obtained with it. Control apparently was obtained for 3 weeks with the other seed treatments. At 5 weeks the greater population in the toxaphene treatment was due to a thrips control dosage being used which was too low to effect boll weevil control.

In experiment 2 boll weevils were found in the treated seed plots 4 weeks after plant emergence. The higher population in the 3911 plot was due to its proximity to hibernation quarters. The lower population in the check as in experiment 1 was due to severe thrips injury to plants.

In experiment 3 no weevils were found in the plots planted with seed treated with 1 pound of 3911 until 5 weeks after plant emergence but they occurred in the check after 2 weeks. There was less thrips injury in this check than in the other experiments and weevils presumably had free choice to enter either plot. The high population in the treated seed plot after 6 weeks was due to influx of weevils because of increased fruiting over check resulting from a considerably lower cotton fleahopper infestation. Apparently no weevils were being killed at that time.

In experiment 4, results varied in the various replicates, although some control of weevils was indicated 4 weeks after plant emergence, which substantiated the control trend established in the cage tests. This control was not so good as that obtained with the toxaphene spray treatments, especially that indicated at 5 and 6 weeks after weevil dosages were used.

Cotton Fleahopper.—Plants were caged at various intervals after plant emergence in the plots planted with seed treated with 1 pound of 3911 and untreated check in experiment 4. Cotton fleahopper adults collected from evening primrose were introduced in the cages and control percentages were determined by Abbott's formula. When fleahoppers were introduced 17 days after plant emergence, the control after 1 day was 80.6%. When they were introduced 21 days after plant emergence, control after 3 days was 46.6%. When they were introduced 28 days after plant emergence, control after 7 days was only 5%.

In experiment 1 adult cotton fleahoppers occurred in small numbers in all treated plots 2 weeks after plant emergence (table 3). After 3 weeks no nymphs were found in the treated seed plots but a few were found in the other treatments. After 4 weeks nymphs occurred in all treatments, and total populations were significantly lower in the toxaphene spray treatment than in each of the other treatments. After 5 weeks the infestation increased to high levels for that stage of plant growth in the treated seed plots and the infestation in the toxaphene treatment was significantly lower than in each of the other treatments. Control with the 1-pound dosage of 3911 was significantly better than with 12008. The nymphal population was considerably less in the 1-pound 3911 plots than in the 0.5-pound plots and significantly less than in the 12008 and check plots. On June 8 a windstorm with a beating rain reduced the infestation considerably in the untreated check and in the treated-seed plots. When an average of the 6 weekly records was considered, toxaphene spray gave significantly better control than each of the other treatments and one pound 3911 gave significantly better control than 12008.

In experiment 2 adult cotton fleahoppers occurred in all plots during the first 2 weeks after plant emergence, but fewer in the treated seed plots than in the check. After 4 weeks nymphs occurred in all plots with lower total populations in the treated seed plots than in the check despite the severe thrips injury in the latter treatment. After 5 weeks the infestation reached injurious levels in all plots but nymphal populations were considerably lower in the 3911 than in the 12008 plots.

In experiment 3 the infestation remained very low in the 3911-treated-seed plot for 6 weeks after plant emergence, but it reached injurious levels in the check. It is concluded that adults were controlled during the first 4 weeks in the seed treatment thus preventing a build-up of populations. Adult migration into the experiment was apparently light after that period. In experiment 4 infestations were too light to evaluate control.

Table 3.—Effectiveness of Am. Cyanamid 3911 and 12008 as seed treatments against cotton fleahoppers in field experiments, Waco, Tex., 1955.

Treatments	Pounds of Toxicant per Acre	Number of Fleahoppers per 100 Terminals on Specified Weeks After (Plant Emergence)						
		1	2	3	4	5	6	Average
		Experiment 1						
3911	0.5	—	1.1	2.2	5.8	32.5	7.8	9.9
	1.0	—	1.4	1.7	4.6	26.2	5.8	7.9
12008	1.0	—	1.8	2.2	4.8	39.2	9.5	11.5
Toxaphene spray	1.0[a]	—	1.7	2.8	1.8	5.2	2.5	2.8
Untreated		—	1.2	1.8	5.7	34.5	6.5	9.9
L.S.D. at 5% level		—	—	—	2.0	9.8	3.4	2.0
		Experiment 2						
3911	1.0	0.2	1.5	2.1	2.6	18.8	14.2	6.6
12008	1.0	.4	2.8	1.2	3.3	29.8	14.5	8.7
Untreated		.6	5.9	4.0	6.0	20.0	19.2	9.3
		Experiment 3						
3911	1.0	0	0.3	1.9	2.4	3.0	0.8	1.4
Untreated		1.0	2.9	8.5	5.9	4.5	21.8	7.5

[a] Average of 6 weekly applications after plant emergence.

Table 4.—Height of cotton plants reflecting thrips control obtained with Am. Cyanamid 3911 and 12008 as seed treatments in field experiments, Waco, Tex., 1955.

Treatments	Pounds of Toxicant per Acre	Height of Plants (Inches) on Specified Weeks After (Plant Emergence)				
		3	5	6	7	Average
		Experiment 1				
3911	0.5	5.2	11.4	—	21.0	12.5
	1.0	5.5	11.2	—	21.7	12.8
12008	1.0	5.3	11.1	—	21.4	12.6
Toxaphene	1.0[a]	5.5	11.9	—	21.5	13.0
Untreated		4.6	10.3	—	20.2	11.7
L.S.D. at 5% level		0.5	0.8	—	—	0.6
		Experiment 2				
3911	1.0	5.5	12.8	—	22.8	13.7
12008	1.0	5.1	12.4	—	21.7	13.0
Untreated		3.9	9.2	—	19.0	10.7
		Experiment 4				
3911	1.0	5.0	9.5	14.6	—	9.7
Toxaphene spray	1.05[b]	4.4	9.1	14.1	—	9.2
Standard toxaphene spray	1.25[c]	3.7	6.9	13.3	—	8.0
Untreated		3.8	6.4	11.7	—	7.3

[a] Average of 6 weekly applications after plant emergence.
[b] Average of 5 weekly applications after plant emergence.
[c] Average of 3 weekly applications beginning 3 weeks after plant emergence.

Plant Growth and Fruiting.—Control of thrips obtained with the seed treatments was reflected in plant growth. In experiment 1 plants in all the treated plots were significantly taller than in the check 3 weeks after plant emergence and in all but the 12008 treatment after 5 weeks (table 4). There was no significant difference in height of plants among treatments after 7 weeks. In the large-plot experiments differences in plant growth were considerably greater and were apparent for a much longer period of time. Differences in plant appearance in one replicate of experiment 4 five weeks after plant emergence may be noted in figures 1 and 2.

Form counts made 6 weeks after plant emergence indicated accelerated fruiting in seed treatments in all experiments. There was no appreciable difference between the 1-pound dosage of 3911 and toxaphene spray except when sufficient boll weevils and cotton fleahoppers were present to cause loss of fruit after the fifth week. Three bloom records were made covering a 2-week period ending at the blooming peak (table 5). In experiment 1 there were significantly more blooms per acre in the treated seed plots and toxaphene spray than in the untreated check. There were significantly more blooms in toxaphene spray than in the seed treatments because it gave better cotton fleahopper control.

In experiment 2 thrips control was reflected by increased blooming in the treated seed plots over check although fleahopper injury was about the same. All plots received one application of toxaphene spray for cotton fleahopper and boll weevil control 6 weeks after plant emergence. In experiment 3 increased blooming occurred in the 3911-treated-seed plot over check reflecting thrips and cotton fleahopper control.

In experiment 4 the difference between the 3911 seed treatment and untreated check was not so great as in the other experiments, because of heavy overwintered boll weevil populations during the early fruiting period

Fig. 1.—Field view showing plants grown from untreated seed on left of center and those grown from 1 pound 3911 treated seed right of center. Photographed May 31, 1955, five weeks after plant emergence.

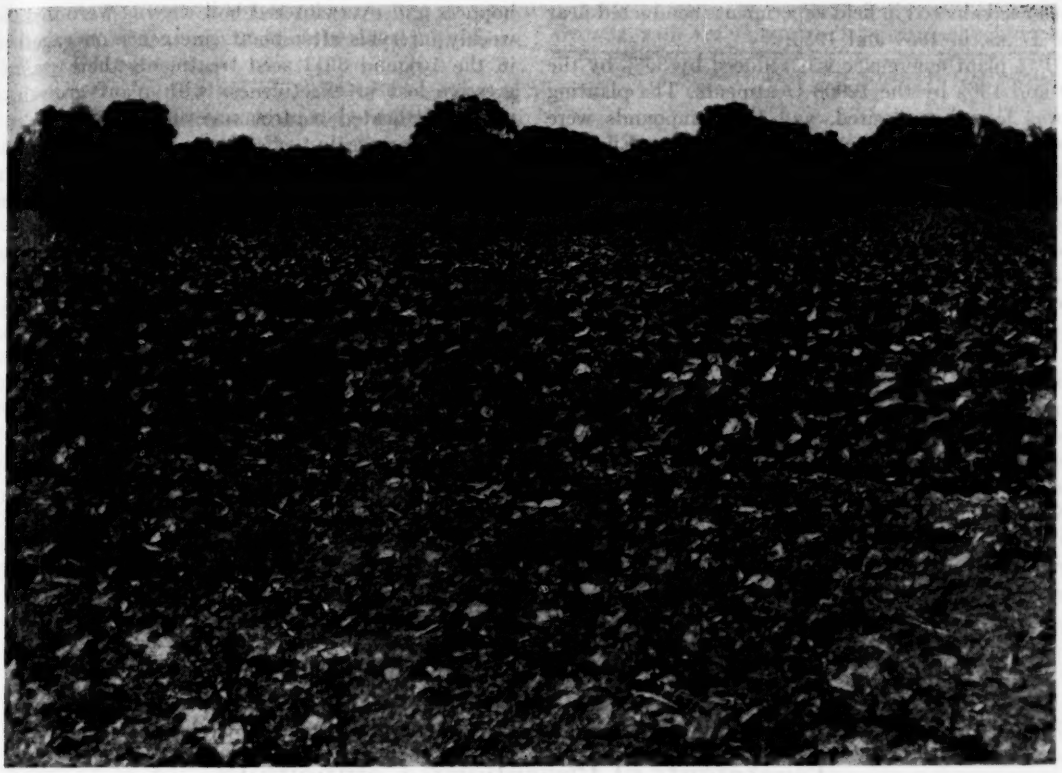

FIG. 2.—Across the field view, showing appearance of plants grown from untreated seed in foreground and those grown from 1 pound 3911 treated seed in background. Photographed May 31, 1955, five weeks after plant emergence.

in one replicate. The difference in favor of the toxaphene treatments was due to boll weevil control.

Late-Season Insect Control and Yields.—Each of the experiments except No. 3 received late-season treatment for boll weevil and bollworm control. It and one replicate of experiment 4 were not favored with July rains. The others were so favored, and near maximum production was achieved.

In experiment 1 the 1-pound 3911 seed treatment and toxaphene spray produced significantly more seed cotton per acre than the untreated check. The 1-pound dosage of 3911 gave a significantly higher yield than the 0.5-pound dosage. Yield records were not obtained in experiment 3 because of poor stand and effects of cotton root rot. Yields in the various treatments of the other experiments did not differ significantly.

CONCLUSIONS.—Results obtained in the experiments indicate that Am. Cyanamid 3911 applied as a seed treatment at 1-pound per acre will afford protection against thrips, cotton aphid, and possibly serpentine leaf miner through the early-season insect control period in central Texas. It does not remain effective against cotton fleahoppers and boll weevils long enough to give such good control of these pests as is obtained with the conventional early-season control program. If the seed treatment is used, the plantings should receive the last conventional early-season application for this control at the time it is normally scheduled in the area.

SUMMARY.—The effectiveness of Am. Cyanamid 12009, 12008, and 3911 employed as seed treatments against thrips (*Frankliniella sp.*), cotton aphid (*Aphis gossypii* Glov.), serpentine leaf miner (*Liriomyza pusilla* (Meig.)), cotton fleahopper (*Psallus seriatus* (Reut.)) and the overwintered boll weevil, (*Anthonomus grandis*

Table 5.—Bloom counts in cotton field experiments in which Am. Cyanamid 3911 and 12008 were employed as seed treatments, Waco, Tex., 1955.

TREATMENTS	POUNDS OF TOXICANT PER ACRE	BLOOMS PER ACRE ON				
		June 22	July 1	July 8	July 15	Average
		Experiment 1				
3911	0.5	—	2062	6285	13358	7235
	1.0	—	2558	6255	13312	7375
12008	1.0	—	1598	6008	14205	7270
Toxaphene spray	1.0[a]	—	3270	9262	15075	9202
Untreated		—	1275	4732	10718	5575
L.S.D. at 5% level		—	—	—	—	1150
		Experiment 2				
3911	1.0	—	3720	7950	15480	9050
12008	1.0	—	2900	10080	20190	11057
Untreated		—	1500	6390	15120	7670
		Experiment 3				
3911	1.0	680	3500	8030	—	4070
Untreated		240	2000	6380	—	2873
		Experiment 4				
3911	1.0	73	2540	8267	—	3627
Toxaphene spray	1.05[b]	290	3457	12220	—	5322
Standard toxaphene spray	1.25[c]	410	3730	11753	—	5298
Untreated		70	1087	10130	—	3396

[a] Average of 6 weekly applications after plant emergence.
[b] Average of 5 weekly applications after plant emergence.
[c] Average of 3 weekly applications beginning 3 weeks after plant emergence.

Boh.), was evaluated in field experiments conducted near Waco, Texas, in 1954 and 1955.

In 1954 plant emergence was reduced by 39% by the 12009 and 13% by the 12008 treatments. The planting rate was less than desired, and the compounds were applied at slightly more than ½ pound per acre. Effective thrips control was afforded for 3½ weeks and some control for an additional week after plant emergence. Am. Cyanamid 12008 was slightly more effective than 12009. Cotton aphid control was obtained for 4½ weeks.

In most of the 1955 experiments the treated seed was planted under good germinating conditions and 1-pound per acre dosages of 3911 and 12008 did not adversely affect plant emergence. Emergence was reduced in one planting of 3911-treated seed following a heavy rain on the day after planting. Leaf miner infestations failed to develop to any extent on cotyledons in the seed treatments. Thrips control was obtained with the seed treatments for 5 weeks after plant emergence and infestations approached that of the checks after 6 weeks. The 1-pound dosage of 3911 compared favorably with an insecticidal treatment applied at weekly intervals through the early-season control period. When adult cotton fleahoppers and overwintered boll weevils were installed at weekly intervals after plant emergence on caged plants in the 1-pound 3911 seed treatment, there was a progressive loss in effectiveness with plant growth. Field records indicated control was obtained 4 weeks after plant emergence but effectiveness did not persist long enough for control to be comparable with that obtained with a conventional early-season insecticidal treatment.

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House Fly Control in Dairy Barns with Residual Treatments of Phosphorus Compounds[1]

JOHN W. KILPATRICK and H. F. SCHOOF

Since 1952 the only insecticides showing promise of controlling chlorinated-hydrocarbon-resistant house flies have been the organophosphorus compounds. Hansens & Bartley (1953) showed a 1% Diazinon-1% methoxychlor formulation to provide house fly control up to 30 days. Wingo (1954) reported a residual effectiveness of 5 to 6 weeks against *Musca domestica* with each of two applications of 1% Diazinon suspensions in dairy barns in Missouri. Hoffman & Cohen (1954) tested Diazinon, Chlorthion, Bayer 21/199, and Bayer L 13/59 at varying dosages with sugar and/or piperonyl butoxide or sulfoxide. The only combination showing residual effectiveness at the 28-day level was the Diazinon:piperonyl butoxide:sugar suspension formulation at the rate of 100:200:200 mg./sq.ft.

Kilpatrick & Schoof (1954) in Georgia reported that NPD at the rate of 100 mg./sq.ft. gave 4 to 5 weeks of satisfactory house fly control, whereas malathion-sugar formulations at 100 and at 200 mg. of toxicant/sq.ft. yielded good fly control for 1 to 3 weeks. In New Jersey, Hansens *et al.* (1955) obtained house fly control with residual treatments of malathion, Chlorthion, and Am. Cyanamid 4124 for periods of 17 to 82 days. Dahm & Raun (1955) in Iowa also reported varying degrees of house fly control with Am. Cyanamid 4124, Chlorthion, Diazinon, Pirazinon, and parathion.

This paper presents the results from field tests with six organophosphorus compounds during 1954 and 1955 for the control of multi-resistant house fly populations in dairy barns.

METHODS.—All tests were conducted at dairies in the Savannah, Ga, area where each dairy possessed a herd of 100 to 300 animals. The insecticides were applied to the interior surfaces of the milking barns, calf pens, and other animal shelters at 100 p. s. i. pressure with a power sprayer equipped with flat spray nozzles. Spot treatments were limited-coverage applications to those locations observed as being the more preferred fly resting surfaces, a coverage generally about 50% of that obtained with complete treatments.

Chlorthion, Am. Cyanamid 4124, and Diazinon (as emulsions and suspensions) and Bayer L 13/59 (as a water solution) were tested at dosages of 100 mg. of toxicant and 250 mg. of sugar/sq. ft. Malathion (emulsions and suspensions) was sprayed at the rate of 200 mg. of toxicant and 500 mg. of sugar/sq. ft. Bayer 21/199 (emulsion) was sprayed at a rate of 50 mg./sq. ft. alone and in combination with 50 mg. of DDT/sq. ft. All emulsions were prepared from xylene concentrates of 25 to 57% strength.

Appraisal of the efficacy of the treatments was obtained by making weekly grill counts (Scudder 1947) of the house flies at each dairy. Five counts were made at each of the four general locations (stanchions, building entrances, barn floors, and feed rooms) known to be attractive to high fly populations. The weekly index used is the average of the maximum count from each of these

[1] From the Communicable Disease Center, Public Health Service, U. S. Department of Health, Education, and Welfare, Savannah, Georgia. Accepted for publication June 21, 1956.

four locations. Fly indices below an average of 10 were considered as excellent fly control; those below 20 as satisfactory fly control.

RESULTS.—During 1954 one dairy ("Be") was treated with a malathion emulsion and one dairy ("Ro") with a malathion suspension (table 1). At both dairies pretreatment fly indices were at relatively high levels but the treatment produced sharp immediate reductions in the fly populations. However, at dairy "Ro" treated with the suspension, fly population indices reflected satisfactory control levels only during the initial week after treatment, control being absent after that week. At dairy "Be" treated with the emulsion, fly indices dropped to essentially zero one week after treatment and remained at excellent fly control levels for the ensuing 4 weeks. During the eighth week, sharply increased indices were obvious. A second emulsion treatment at dairy "Be" during mid-July again resulted in an immediate reduction of the fly populations to excellent control levels, which persisted during the following 7 weeks. Subsequently, fly populations increased rapidly in a manner paralleling the trends with the May treatment.

To determine if the poor results with the suspension were due to the formulation or to the dairy involved, the same two dairies ("Be" and "Ro") received malathion-sugar treatments in 1955, except that the formulations used in each were reversed, i.e. dairy "Ro" received the emulsion, dairy "Be" the suspension treatment. Substantial reductions of pretreatment fly indices were again noted at both dairies immediately after treatment. At dairy "Be" (suspension) fly control ranged between excellent and satisfactory for 8 weeks. However, at dairy "Ro" (emulsion treatment) satisfactory fly control was not achieved during the initial posttreatment week, although satisfactory fly control levels were obtained for the subsequent 2 weeks, followed by gradually rising fly indices. The prolonged efficacy of the suspension treatment and the limited residual action of the emulsion application in 1955 is in direct contrast to the 1954 data with the two formulations. Since either formulation was effective at dairy "Be" and relatively ineffective at dairy "Ro" the difference in results is considered a reflection of the dairy involved rather than the formulation employed.

Table 1.—House fly control in dairy barns near Savannah, Georgia with malathion residual bait sprays.[a]

Weeks After Treatment	Fly Counts—Three-Week Moving Average				
	1954			1955	
	Dairy "Be" Emulsion	Dairy "Ro" Suspension	Dairy "Be" Suspension	Dairy "Ro" Emulsion	
−3	47	67	69	70	110
−2	139	159	122	84	89
−1	277	274	189	122	140
0	285	37	228	180	240
1	1	3	18	11	22
2	2	4	28	9	15
3	3	3	31	9	18
4	5	1	37	12	27
5	8	1	54	15	28
6	11	2	81	20	40
7	19	5	96	20	52
8	67	16	128	14	49
9	159	33	178	26	62
10	274	78	181	67	76
11	371	200	138	102	80
Date treated	May 5	July 22	May 6	May 5	May 4

[a] Applied at the rate of 200 mg. per sq. ft., plus 500 mg. of sugar per sq. ft.

Table 2.—House fly control in dairy barns near Savannah, Georgia, with Am. Cyanamid 4124 residual bait sprays.[a]

Weeks	Fly Counts—Three-Week Moving Average					
	1954			1955		
	Dairy "Ba" Suspension	Dairy "H" Emulsion	Dairy "Rg" Suspension	Dairy "T-1" Emulsion	Dairy "VW" Suspension	
Before treatment						
3	162	99	34	—	—	
2	199	125	48	58	—	43
1	196	162	101	89	915	83
0	175[b]	139[b]	160[b]	110[c]	168[c]	91[b]
After treatment						
1	1	4	4	3	20	3
2	1	3	4	5	28	4
3	2	3	4	9	43	5
4	1	2	3	11	83	7
5	1	2	2	19	91	13
6	1	3	2	27	—	19
7	2	4	2	21	—	21
8	—	—	10	26	—	27
9	—	—	23	41	—	32
Date treated	Aug. 18	Aug. 18	July 2	May 10	May 10	June 13

[a] Applied at the rate of 100 mg. per sq. ft., plus 250 mg. per sq. ft. sugar.
[b] Complete treatment.
[c] Spot treatment.

In July 1954, Am. Cyanamid 4124 was applied as a suspension at dairy "Rg" (table 2). A spectacular reduction in the fly populations was obtained and excellent control levels were maintained for 8 weeks. Gradual but definite increases in fly populations were evident during the following weeks. In August 1954, two additional dairies were treated; one with an emulsion (dairy "H"), the other (dairy "Ba") with a suspension. Posttreatment fly indices at both dairies reflected excellent control for 7 weeks following which cool weather prevented further evaluations. At the emulsion-treated dairy, indices remained below an average of five flies per grill count while those at dairy "Ba" did not exceed an average of two flies per grill count.

During May 1955, two dairies received spot treatments of Am. Cyanamid 4124, one with an emulsion (dairy "T-1"), the other with a suspension (dairy "VW"). The emulsion treatment provided excellent fly control for 3 weeks, succeeded by 2 weeks of satisfactory control (table 2). As fly populations began increasing, the owner began using his own control measures and evaluation of the treatment was discontinued. The fly indices at the dairy receiving the spot suspension treatment never reached less than 20 flies at any time and satisfactory control was present for only 1 week. However, dairy "VW" displayed a much higher pretreatment fly index than dairy "T-1" (grill count 1682 versus 110). During late June, a complete re-treatment with a suspension formulation at dairy "VW" provided 4 weeks of excellent control, followed by 2 weeks of satisfactory control. Although fly indices at this dairy increased beyond control levels, they did not approach pretreatment densities.

Chlorthion-sugar as an emulsion was applied to one dairy (dairy "T-1") in July 1954. Excellent fly control was obtained initially and maintained for 8 weeks after treatment (table 3).

Table 3.—House fly control in dairy barns near Savannah, Georgia, with Chlorthion residual bait sprays.[a]

	Fly Counts—Three-Week Moving Average			
	1954	1955		
Weeks	Dairy "T-1" Emulsion	Dairy "Ba" Emulsion	Dairy "W" Suspension	Dairy "Z" Emulsion
	Before treatment			
3	69	64	42	92
2	72	99	63	102
1	84	146	92	154
0	121[b]	186[b]	115[b]	206[c]
	After treatment			
1	5	3	1	6
2	4	3	1	6
3	2	3	3	4
4	2	5	7	4
5	2	6	11	3
6	2	14	13	2
7	4	24	16	2
8	7	21	23	3
9	18	20	22	3
10	13	21	15	4
11	27	—	12	4
12	—	24	9	4
13	—	33	7	4
14	—	25	8	4
15	—	15	8	4
16	—	21	9	5
17	—	22	9	7
Date treated	July 20	June 8	June 8	June 8

[a] Applied at the rate of 100 mg. per sq. ft., plus 250 mg. per sq. ft. sugar.
[b] Complete treatment.
[c] Spot treatment.

In June 1955 Chlorthion was tested at three dairies. One dairy received a complete treatment with an emulsion (dairy "Ba"); one a complete treatment with a suspension (dairy "W"), and one a spot treatment with an emulsion (dairy "Z"). The complete emulsion treatment and the complete suspension treatment provided excellent house fly control for 4 to 5 weeks (table 3). With the exception of 2 weeks, the suspension application gave satisfactory results for the balance of the season. Although the emulsion formulation did not give satisfactory control for the same period, indices remained in the range of 20 to 30 specimens. Excellent control was obtained from the spot emulsion treatment for the entire 17 weeks. However, even though pretreatment indices at dairy "Z" were high, the reproduction potential therein was low and this factor is considered responsible for the extended excellent control obtained.

Diazinon emulsions and suspensions were tested in 1954 at two dairies. The suspension formulation applied to dairy "Bg" in July produced a spectacular and immediate reduction of the fly populations to an average of less than one house fly per grill count (table 4). Excellent fly control was maintained for 8 weeks. The Diazinon emulsion applied to dairy "W" 3 weeks after the suspension treatment of dairy "Bg" produced equally effective reductions in fly populations, excellent control levels being maintained for 8 weeks, followed by 3 weeks of satisfactory control.

The treatments with Bayer 21/199 alone (dairy "Mo") and combined with DDT (dairy "VW") did not provide satisfactory fly control even though marked reductions of fly indices were obtained with both formulations (table 4).

A Bayer L 13/59-sugar solution applied to one dairy (dairy "Be") in August 1955, gave 6 weeks of excellent fly control, followed by a single week of satisfactory abatement (table 4).

Discussion.—In the 1954 tests, emulsions of malathion, Diazinon, Am. Cyanamid 4124, and Chlorthion with a sugar additive provided excellent fly control for periods of 5 to 8 weeks. Similar results also were obtained with suspensions of Diazinon and Am. Cyanamid 4124. Only malathion suspension at 5% with a sugar additive failed to give satisfactory results. However, by reversing the dairies receiving the malathion emulsion and suspension treatments in 1955 it was shown that the status of control achieved also could be reversed. This reversal occurred even though the fly potentials at the two dairies were considered to be of similar magnitude.

It was assumed that during 1954 the 5 to 8 weeks of excellent control achieved with the various formulations reflected a residual efficacy beyond that usually encountered with organic phosphorus compounds, resulting from the effect of adverse (dry) weather conditions which retarded the rapid development of successive fly populations. Consequently, under normal weather conditions, these formulations were considered as probably being effective for shorter periods of time.

In 1955 weather conditions were conducive for fly production. Chlorthion produced 5 weeks of excellent fly control versus 8 weeks in 1954, while Am. Cyanamid 4124 provided only 4 weeks of excellent fly control versus 8 weeks during 1954. These data support the assumption

Table 4.—House fly control in dairy barns near Savannah, Ga., treated with five different phosphorus formulations.

	Fly Counts—Three-Week Moving Average				
	1954				1955
	Dairy "W"	Dairy "Bg"	Dairy "VW"	Dairy "Mo"	Dairy "Be"
	Formulation				
	Diazinon: Sugar Emulsion	Diazinon: Sugar Suspension	21/199: DDT Emulsion	21/199 Emulsion	L 13/59: Sugar Solution
	Dosage				
Weeks	100:250 mg./sq. ft.	100:250 mg./sq. ft.	50:50 mg./sq. ft.	50 mg./sq. ft.	100:250 mg./sq. ft.
	Before treatment				
2	52	67	251	265	102
1	59	126	575	350	110
0	77	173	106	371	129
	After treatment				
1	1	1	25	51	3
2	1	3	26	36	2
3	1	5	32	24	3
4	2	4	55	31	4
5	2	2	101	51	6
6	3	1	187	64	10
7	3	3	204	58	17
8	3	9	—	89	24
9	11	16	—	133	28
10	13	24	—	—	—
11	17	30	—	—	—
12	24	27	—	—	—
Date treated	July 14	June 29	June 17	June 17	Aug. 8

of a reduced efficacy for the 1954 treatments under normal weather conditions and indicate that the effectiveness of short-lived compounds such as the organophosphorus materials may vary several weeks, depending on the influence of weather conditions on fly potential. However, both compounds, although less effective in 1955, still yielded satisfactory control for 6 to 7 weeks.

Previous laboratory tests with Bayer 21/199 had shown that emulsions of this insecticide applied to plywood panels at 50 mg./sq.ft. alone or combined with DDT at 50 mg./sq.ft. were effective for considerable periods of time. The ineffective results obtained with Bayer 21/199 in the two dairies presumably were due to the effects of weathering upon the residues. Laboratory tests had indicated this possibility when deposits exposed to direct sunshine and rain lost their toxicity to house flies after only 1 week.

The dosage of 200 mg./sq.ft. with malathion was used to determine if such deposits would produce extended residual action. Although this dosage yielded 7 to 8 weeks of satisfactory control at certain dairies, it did not provide adequate control at other sites. Since it was shown (Kilpatrick & Schoof 1954) that malathion at 100 mg./sq.ft. gave adequate control for 1 to 3 week periods, the use of the heavier dosage does not appear to be warranted. Based on dosage and the duration of residual action, the efficacy of malathion apparently is below that of Chlorthion, Am. Cyanamid 4124, and Diazinon.

SUMMARY.—Residual application of malathion-sugar formulations in dairies at a rate of 200 mg. of malathion and 500 mg. of sugar/sq.ft. gave 1 to 7 or 8 weeks of satisfactory house fly control in 1954–55. In the same period treatments of Chlorthion and Am. Cyanamid 4124 at 100 mg./sq.ft. plus a sugar additive (250 mg./sq.ft.) provided satisfactory house fly control for 6 to 8 weeks in 1954 and 1955. Diazinon, tested only in 1954 at a similar dosage gave 9 to 11 weeks of satisfactory control. Unfavorable weather conditions for house fly propagation in late 1954 were responsible for the extended excellent control levels present in that year. Although a lower degree of control was apparent in 1955, Am. Cyanamid 4124 and Chlorthion produced 6 to 7 weeks of satisfactory control respectively.

Bayer 21/199 alone at 50 mg/sq.ft. and in combination with DDT (50 mg./sq.ft.) did not provide satisfactory fly control at two dairies in 1954. A single application of Bayer L 13/59-sugar formulation at 100 mg. of L 13/59 and 250 mg. of sugar/sq.ft. provided 7 weeks of satisfactory control.

Spot treatments (50% of normal coverage) at two dairies with Am. Cyanamid 4124 resulted in poor control at one dairy and 5 weeks of satisfactory control at the second.

By reversing the type of malathion formulations used at two dairies during successive years, it was shown that the locale rather than the formulation was the factor responsible for the difference in results.

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Cabbage Looper Control[1]

GEORGE P. WENE, *Texas Agricultural Experiment Station, Weslaco*

The cabbage looper, *Trichoplusia ni* (Hbn.), is one of the most difficult insects to control in the Lower Rio Grande Valley of Texas. Farmers are hesitant about conducting a regular control program because the price of cabbage fluctuates greatly, and as a result they apply treatments only when the cabbage loopers are seen. Most growers start their control work on mature cabbage loopers. Because of the difficulty encountered in controlling large cabbage loopers a series of experiments was conducted in order to evaluate insecticides for control of large or mature loopers, and to find some insecticides which could be used within 30 days prior to the harvesting of the cabbage. Previous work by Wene (1954) and Wene & Otey (1955) showed that applications of dust concentrations of 20% toxaphene and 2% endrin resulted in excellent control of the cabbage looper.

PROCEDURE.—The plots in all but the large scale experiment were 0.02 acre in size, with each treatment replicated three times. The treatments shown in the tables were applied with rotary hand dusters. In the large scale experiment the insecticidal dusts were applied with a tractor-drawn duster to single blocks 10 acres in size with the untreated area being only 1 acre.

In the replicated experiments the efficiency of the various materials was determined by selecting 10 plants at random from each plot and counting the live cabbage loopers at various time intervals after treatment. In the tractor-duster experiment the live cabbage loopers were counted on 30 plants selected at random from each plot.

DISCUSSION OF RESULTS.—The 1954 experiment was

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Table 1.—Control of cabbage loopers on young cabbage with various insecticidal dusts. 1954.

Treatments (20 Pounds per Acre)	Cabbage Loopers per 10 Plant Sample After			
	1 Day		7 Days	
	Number	Per Cent Control	Number	Per Cent Control
1.5% endrin	0.7	91	0.0	100
20% toxaphene	1.0	87	0.0	100
5% malathion	2.0	74	0.7	83
5% DDT+2% parathion	1.7	70	0.0	100
5% DDT+3% malathion	0.0	100	0.0	100
Untreated	7.7	—	4.0	—

conducted during a period when the maximum daily temperature never was above 75 degrees F. The cabbage plants had not yet started to form heads and all of the cabbage looper larvae were small and feeding on the underside of the outer leaves. The data in table 1 show that 20% toxaphene, 1.5% endrin, and 5% DDT combined with either 2% parathion or 3% malathion gave excellent control. A 5% malathion dust gave commercial control but was less effective than the other dusts tested as can be seen by the data.

The 1955 experiments were conducted during a period when the maximum temperature never exceeded 70 degrees F. The cabbage heads were almost mature. The cabbage loopers varied in size with approximately 50% of the larvae being half grown or larger. The larger larvae were observed feeding on the base of the leaves adjacent to the head. The data in table 2 show that 20% toxaphene dust was the most effective insecticide. A standard mixture of 0.9% piperonyl butoxide with 0.06% pyrethrins gave good control but was not as effective as toxaphene. The data in experiment 1 of table 2 show that raising the poundage per acre of the piperonyl butoxide—pyrethrins dust did not give any better control. Increases in the percentage of piperonyl butoxide and pyrethrins in the dust did not increase the effectiveness of the dust. In the tractor-duster experiment the data taken 1 day after treatment show that 0.9% piperonyl butoxide plus 0.06% pyrethrins was more effective at the 40-pound rate than at 20 pounds. However, data taken 6 days after treatment show both rates of application to be about equal in effectiveness.

Table 3.—The effectiveness of various insecticidal dusts in controlling large cabbage loopers. 1956.

Treatments (30 Pounds per Acre)	Looper per Plant After			
	1 Day		5–6 Days	
	Number	Per Cent Control	Number	Per Cent Control
Experiment 1				
0.9% piperonyl butoxide+0.06% pyrethrins	0.8	69	0.9	63
0.1% pyrethrins+0.1% N-octyl bicycloheptene Dicarboximide	0.8	69	0.9	63
0.1% pyrethrins (Impregnated)	1.2	54	1.3	46
Untreated	2.6	—	2.4	—
Experiment 2				
7.5% malathion	1.6	58	0.8	72
0.75% rotenone+2% malathion	0.9	76	0.9	69
1% rotenone	2.0	43	1.8	38
0.9% piperonyl butoxide+0.06% pyrethrins	1.6	58	1.0	66
20% toxaphene	1.8	53	0.8	72
Untreated	3.8	—	2.9	—
Experiment 3				
2% Shell OS 2046	1.5	58	0.6	79
1.5% Bayer 17147	2.5	31	1.3	54
20% toxaphene	1.9	46	0.7	75
Untreated	3.6	—	2.8	—

In 1956 experiments were conducted during a period when the maximum daily temperatures were above 90 degrees F. The cabbage in the test fields was mature and ready for harvesting. Most of the cabbage loopers were two-thirds grown or larger. The data recorded in experiment 1, shown in table 3, show that 0.9 % piperonyl butoxide with 0.06% pyrethrins was more effective than 0.1% pyrethrins. Addition of the activator, N-octyl bicycloheptene dicarboximide, increased the effectiveness

Table 2.—The effectiveness of a mixture of piperonyl butoxide with pyrethrins in controlling cabbage loopers, 1955.

Treatments	Pounds per Acre	Cabbage Loopers per Plant After			
		1 Day		5–6 Days	
		Number	Per Cent Control	Number	Per Cent Control
Experiment 1: Hand Dusters					
2.25% piperonyl butoxide+0.15% pyrethrins	25	0.8	70	0.4	67
0.9% piperonyl butoxide+0.06% pyrethrins	38	.6	77	.5	58
0.9% piperonyl butoxide+0.06% pyrethrins	50	.7	74	.7	42
20% toxaphene	25	.4	85	.1	92
Untreated	—	2.7	—	1.2	—
Experiment 2: Hand Dusters					
0.9% piperonyl butoxide+0.06% pyrethrins	34	1.2	68	0.5	79
0.9% piperonyl butoxide+0.06% pyrethrins	13	3.1	16	1.4	42
20% toxaphene	35	2.2	41	.2	92
Untreated	—	3.7	—	2.4	—
Experiment 3: Tractor Duster					
0.9% piperonyl butoxide+0.06% pyrethrins	20	1.8	51	0.6	75
0.9% piperonyl butoxide+0.06% pyrethrins	40	.7	81	.5	79
Untreated	—	3.7	—	2.7	—

of 0.1% pyrethrins dust. This activated pyrethrins dust and the piperonyl butoxide-pyrethrins dust are effective enough to be used close to the harvesting period as it leaves no poisonous residue as toxaphene or endrin does.

Rotenone has been recommended in the past for the control of the cabbage looper. The data in table 3 show that rotenone gave very little control. A combination of 0.75% rotenone with 2% malathion was just as effective as 0.9% piperonyl butoxide with 0.06% pyrethrins. A dust concentration of 7.5% malathion was just as effective as the 20% toxaphene dust. Shell OS 2046, proved to be slightly more effective than 20% toxaphene whereas Bayer 17147, used at a 1.5% concentration, failed to give commercial control.

A 20% toxaphene dust was very effective in controlling small cabbage loopers during cool weather as can be seen by the data in table 1. During hot weather the effectiveness of toxaphene against larger cabbage looper larvae decreased as can be seen by the data of table 3. This agrees with the field observations that showed that toxaphene lost some of its effectiveness against cabbage looper when the temperature was above 90 degrees F.

SUMMARY.—Good control of the cabbage looper was obtained with the following insecticidal dusts: 1.5% endrin; 20% toxaphene; 5% DDT with 2% parathion or 3% malathion; 0.9% piperonyl butoxide with 0.06% pyrethrins; 0.1% pyrethrins with 0.1% of the activator, N-octyl bicycloheptene dicarboximide; concentrations of 5 and 7.5% malathion; 0.75% rotenone with 2% malathion; and Shell OS 2046.

The following dusts failed to give commercial control: 1% rotenone; 0.1% impregnated pyrethrins; and Bayer 17147.

The data indicate that the cabbage looper control program should start when the larvae are small because they are easier to kill than when half grown or larger.

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Malathion Spray for Fowl Tick Control[1]

J. L. RODRIGUEZ, JR., and L. A. RIEHL, *University of California Citrus Experiment Station, Riverside*

The fowl tick or blue bug, *Argas persicus* (Oken), is a relatively uncommon ectoparasitic pest in our modern poultry industry but is widespread in farm and ranch flocks in the Southwestern States. It is encountered occasionally, however, in isolated infestations, and its occurrence on the premises of a poultry establishment has constituted a serious problem because control has been very difficult and expensive.

Argas persicus is indigenous to arid and semiarid areas of the United States and, according to Essig (1926), is found throughout much of California. Since the tick feeds on its hosts at night and leaves them during the day, an infestation may become established and reach serious proportions before it is noticed. Fowls are injured directly by a loss of blood which may result in a decrease in resistance to other parasites or diseases. Repeated feeding by ticks contributes to lower egg production and market weight, and blemishes left on the skin by the feeding ticks result in an economic loss, especially to commercial turkey producers.

The adult female tick is capable of producing as many as 900 ova in a season. Clusters of eggs are deposited in protected places. Available information indicates that the incubation period may range from 10 to 90 days under varying environmental conditions. After hatching, the minute gray hexapodus larvae seek out a roosting fowl for their first blood meal and may remain attached to the host for 3 to 10 days during this initial feeding period. At this stage of their development the larvae are often called seed ticks. At the end of this period the seed tick drops off its host and goes to a suitable hiding place, where digestion and assimilation of the blood meal are followed by moulting. After the first moult, the fourth pair of legs appear. From this stage on, the length of each feeding period is of short duration. After a series of blood meals and moults, the tick reaches maturity. The adult will live and continue to prey on its poultry host for a long period. An important consideration of the nature of this pest is the evidence that the adult can survive as long as 2 years without a blood meal.

Bishopp (1919, 1942) discusses the use of crude petroleum, carbolineum, and creosote oils as combative agents. Stage (1947) reports on treatments using DDT sprays, and Smith (1952) has demonstrated interesting results with dieldren, aldrin, and lindane. BHC, toxaphene, and chlordane used in spray form for fowl tick control are discussed by Hall & Wehr (1953) and in U. S. Dept. Agric. Leaflet No. 382 (1955). Suggestions for the use of the pesticides mentioned, however, include qualifications to the effect that it may be necessary to repeat the treatments at intervals. This lack of complete control with a single application made it desirable to test other chemicals for fowl tick control. Malathion was selected for this purpose.

Vincent *et al.* (1954) have reported effective control of the northern fowl mite by the use of malathion and have presented evidence showing that chickens were not affected deleteriously by direct application of dusts containing as much as 25% malathion, or by the addition of 50 parts per million of malathion to the feed. They also found that the taste of the eggs was not altered.

[1] Paper No. 917, University of California Citrus Experiment Station, Riverside, California. Accepted for publication July 3, 1956. The pesticide chemicals discussed may not be used unless a tolerance has been established or an exemption from the requirement of a tolerance has been granted for each specific use, irrespective of the information contained in this report.

These preliminary findings by Vincent *et al.* (1954) are supported by intensive toxicological studies by March *et al.* (1956) on the fate of malathion in the chicken hen. Malathion was administered to the chickens as (1) 100 p.p.m. of the feed for 15 consecutive days, (2) 112 p.p.m. of body weight by complete external coverage with a single application of 0.5% spray, and (3) 178 p.p.m. of body weight by a single intraperitoneal injection. No gross toxic effects were noted in either the fed or the sprayed hens; even in the birds treated by injection, the effects did not cause prostration. The maximum total residues found in any of the tissues or eggs examined were less than 3% of the concentration received by feed and only 0.1% of this level for sprayed hens; residues decreased rapidly after exposure to malathion was terminated. Evidence indicates that 90% or more of the total residues found are metabolites and degradation products of malathion and that it is probable that the major part of these are relatively nontoxic.

In the early part of May, 1955, a serious chronic infestation of fowl tick was reported in the White Leghorn laying flock of a small chicken ranch near Nuevo, California. The conditions were suitable for field experiments and plans were made to test the efficacy of malathion when applied in aqueous spray to the hen houses.

MATERIALS AND METHODS.—Two pens containing 300 hens each, 1 pen containing 150 hens, and 1 containing 6 hens were included in the tests. Tick-count stations or traps were established at random in each hen house. Each trap consisted of a 3-inch band of adhesive tape which was placed around studs, roost and nest supports, and rafters. The tape was placed so that only 1 edge adhered to the support. The opposite edge of the tape trap was left open. The open end was never more than $\frac{1}{8}$ inch from the surface of the support. When the ticks emerged to feed, they were compelled to crawl under the open end of the tape trap in order to reach the roosting birds. As the tick moved forward, its dorsal surface came in contact with the adhesive side of the tape and it was trapped. Once affixed, the ticks, as well as other bugs which were present, were held securely for counting. The traps were examined on each of 3 successive days prior to treatment and at approximately weekly intervals after treatment, through a period of 100 days. The adhesive tape was replaced with a fresh band in each trap at each count.

Prior to spraying, all hens, eggs, feeders, and watering troughs were removed from the pens. They were not returned until the sprayed buildings were completely dry. No stress was noted in the hens following the malathion house spraying.

Malathion was used in aqueous spray which was mixed and applied by means of power-driven spray equipment utilizing a high-pressure reciprocating-type pump and provided with satisfactory agitation in the spray tank. During treatment of the inside and outside structural portions of the chicken houses, the spray gun was adjusted to give a coarse spray of 300 p.s.i., and this was directed against accessible surfaces at close range to drive the spray deep into the cracks and into crevices under studs, joists, and roofing material. Enough spray was applied to cause runoff. The full force of the spray stream was used to turn and churn the litter and distribute the spray through it; then a spray composed of fine droplets was applied to the surface of the litter.

A complete pen comprised a single treatment unit. One pen was left untreated to serve as a check. Three other pens were treated with malathion at actual concentrations of 1.0%, 0.5%, and 0.25%, respectively, obtained by using the proper amounts of the 57% emulsifiable liquid formulation.

Immediately after the applications, great numbers of ticks swarmed out of hiding, many of them saturated with the spray material. Approximately half an hour after treatment, large numbers of dead or moribund ticks were noted on the floor of the chicken houses and on the ground outside of the foundations. Several small sections of the tar-paper roofs were removed from each house to determine the actual penetration of the spray under the roofing material. On the whole, the area immediately under the tar paper was untouched by the spray. It was observed that a large part of the tick population was hidden in this area. Thus, a considerable number of ticks and eggs were not exposed directly to the spray treatments.

DISCUSSION OF RESULTS.—The results of the treatments are shown in table 1. These data indicate that a single application of 1.0% malathion spray applied to the structural portions of a poultry house will control a fowl-tick infestation very effectively. The numerical reduction in ticks is immediate. Results also suggest that the residual effect of this treatment kills the seed ticks as they leave the hens, and the adult ticks and the newly-hatched larvae as they emerge to feed. The 0.5% and 0.25% sprays gave a good initial kill but failed to afford complete control with a single application. For commercial reasons, the hens were removed from the pens

Table 1.—Effectiveness of single applications of malathion sprays on chicken houses for control of the fowl tick.

COUNTS (DAYS BEFORE AND AFTER TREATMENT)	AVERAGE NUMBER OF TICKS PER TRAP PER COUNT			
	Pen 1[a] (Untreated)	Pen 2[b] (1% Spray)	Pen 3[c] (0.5% Spray)	Pen 4[d] (0.25% Spray)
Before Treatment				
1	10	10.0	16.0	44.0[e]
2	7	27.0	19.0	—
3	3	33.0	0.0	—
After Treatment				
1	21	2.0	1.0	4.0
7	8	12.0	2.0	0.8
14	1	0.3	4.0	0.6
21	12	0.5	1.0	0.3
28	7	0.5	0.3	0.8
35	5	1.0	0.2	2.0
49	5	0.0	0.4	0.3
58	7	0.0	—	—
70	4	0.0	—	—
77	1	0.0	—	—
85	3	0.0	—	—
100	6	0.0	—	—

[a] Area, 900 sq. ft.; hens, 300; traps, 15.
[b] Area, 900 sq. ft.; hens, 300; traps, 12.
[c] Area, 600 sq. ft.; hens, 150; traps, 10
[d] Area, 70 sq. ft.; hens, 6; traps, 10.
[e] Most of these ticks were in the larval stage and had not had the first blood meal.

given these two treatments and weekly counts were discontinued at the end of 50 days.

SUMMARY AND CONCLUSIONS.—Aqueous sprays of malathion, prepared from the 57% emulsifiable liquid formulation, were tested for control of the fowl tick (blue bug), *Argas persicus* (Oken), infesting the White Leghorn laying flock of a small southern California chicken ranch. The sprays were applied by power-driven equipment, under high pressure, to the inside and outside structural portions of the hen houses and to the litter on the floor. Particular attention was given to wetting such areas as those behind wall boards and timbers, under joists, studs, and roofing material, and in cracks. A spray containing 1.0% actual malathion, applied as described, gave complete control of the fowl tick by the 49th day after application, following progressive decrease in the numbers of ticks present.

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The Compatibility of Insecticides and Fungicides Used for the Treatment of Corn Seed[1]

E. W. ARNOLD and J. W. APPLE[2] *Department of Entomology, University of Wisconsin, Madison*

Within the last few years considerable interest has been directed towards protecting germinating corn seed with insecticides as well as fungicides. Germinating corn is attacked by soil-inhabiting fungi and several species of insects. According to Hoppe (1953) various species of *Pythium* are responsible for the major part of corn seed decay in Wisconsin soils. The insects commonly found on germinating corn seed in Wisconsin are wireworms (Elateridae), the seed-corn maggot, *Hylemya cilicrura* (Rond.), and the seed-corn beetles, *Agonoderus lecontei* Chaud. and *Clivina impressifrons* Lec.

Among the problems associated with the use of insecticide-fungicide mixtures, compatibility of the chemicals appears to be of major concern, and few instances of incompatibility have been recorded. Marshall (1951) reported considerable confusion existed in 1948 and 1949 as to the compatibility of certain fungicides and insecticides when used together on fruit trees. In early laboratory tests, Chapman (1950) reported copper sulfate reduced the insecticidal efficiency of DDT. Duffield (1952) observed corn plants grown from seed treated with a dieldrin-thiram mixture 12 weeks before planting produced lower yields than plants from seed treated with dieldrin alone. Neiswander (1955) found malathion, parathion, demeton, and EPN generally compatible with the dithiocarbamate fungicides, but recent work indicates malathion is not compatible with ferbam, at least in dust form. McCellan *et al.* (1955) found malathion had adverse effects on captan when used for control of blackspot on roses.

At the same time, favorable results have been recorded from the use of insecticides in combination with fungicides. Howe & Schroeder (1951) conducted experiments over a 4-year period and demonstrated insecticide-fungicide mixtures on bean seed produced better stands in the presence of pathogenic fungi and insects than when the seed was treated with insecticides alone. Lindane was found to be phytotoxic, but when used with a fungicide the phytotoxicity was reduced. Duffield (1952) found lindane seed treatments on corn to be less injurious when in the presence of thiram. He concluded lindane injury made corn seed more susceptible to seed rot organisms, as was also suggested by Ditman *et al.* (1955) for pea seed. In a summary of fungicide tests, Roberts (1955) reported onion seed pelletized with Tersan 75 or Orthocide 75 and heptachlor or dieldrin resulted in large increases in yield where soil was heavily infested with onion smut fungus and onion maggot *Hylemya antiqua* (Meig.). Ditman *et al.* (1955), working with peas and beans, concluded that cold wet soils favored development of decay organisms and at such times a fungicide is necessary on seed; whereas soils that were cold but drier became infested with seed-corn maggot, and here an insecticide was most beneficial on the seed.

METHODS AND MATERIALS.—Tests were conducted with four insecticides and three fungicides to determine whether fungicidal action was inhibited by the presence of an insecticide. In the laboratory, a test procedure based on the Hoppe, "paper doll" method (1953) was

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[2] Formerly graduate student in Entomology, at present Entomologist with Wisconsin Department of Agriculture, and Professor in Entomology, respectively.

employed. By this method, exaggerated fungal damage to corn seed is obtained by removing the crown of the seed. After being injured, the corn seed (W575) was slurry treated to provide 1 ounce of actual insecticide and 0.75 ounce actual fungicide per bushel of seed. These dosages were obtained from wettable powders containing 75% lindane, aldrin, dieldrin, thiram and captan and wettable powders containing 50% heptachlor and dichlone. Each fungicide was used separately to provide information on normal seed protection, and each insecticide was used in the absence of a fungicide to measure the fungicidal action of the insecticides.

Fifty seeds of a given treatment were imbedded in 80 grams of *Pythium* infested muck soil scattered over a moistened 10″×11″ paper towel. Another moistened towel was placed over the soil and seed and then the entire preparation was rolled into a "paper doll." Three replicates of each treatment were prepared and placed in moisture retaining cans in a 48° F. refrigerator for 10 days. Upon removal from the refrigerator the "paper dolls" were placed in an 85° F. incubator for 3 days. After this exposure, the seedlings were scored on the basis of two points for a normal sprout, and one point for a weak sprout.

Similarly injured and treated seed was planted in muck soil during the spring of 1955 to evaluate the influence of insecticides on fungicides on a practical but less critical basis. Individual plots consisted of 36 seeds planted in 12 hills of one row. Treatments were replicated three times. Response to the seed treatments was measured by means of stand counts and ear harvest. Harvesting was confined to 10 of the 12 hills, thus leaving a "buffer" hill at either end of each plot.

Insect bioassays were conducted to determine the effect of fungicides captan, dichlone and thiram on the toxicity of dieldrin. A non-resistant strain of fruit flies, *Drosophila melanogaster* Meig., obtained from the Department of Genetics, University of Wisconsin, was used as the test insect. The flies were cultured on canned pumpkin as suggested by Bartlett (1951) and employed in a testing technique similar to that proposed by Sun & Pankaskie (1954) for the detection of insecticidal residues. Wettable powder suspensions of dieldrin, dieldrin-fungicide mixtures, and fungicides alone were blended into canned pumpkin purée to provide a toxic food source for the flies. Insecticide-fungicide mixtures were prepared in the ratio of 1 part dieldrin to 0.75 part fungicide and various amounts of this stock suspension were used to provide a range of dosages. About 20 grams of treated pumpkin were placed in a 4-ounce bottle and 25 three-day old CO_2 anaesthetized flies were introduced into the bottles on a paper tray. Four replicates were used per treatment. Mortality was determined 24 hours after the introduction of flies. A fly was considered dead when it was incapable of standing. Dosage mortality data were analyzed by the method of Litchfield & Wilcoxon (1949) to determine LD_{50}s and slope of regression lines.

RESULTS AND DISCUSSION.—Seed treated at the rate of 1 ounce per bushel with dieldrin, heptachlor, lindane or aldrin in combination with 0.75 ounce of captan per bushel provided as good protection from pathogenic soil fungi as when captan was used alone (table 1). Seedling growth was normal from seed treated with thiram and dichlone in combination with the insecticides except for treatments involving 1 ounce of lindane in which case the germination ratings were found to be significantly lower than the rating of the corresponding fungicide-treated seed. This adverse response to lindane may have resulted from direct injury by the insecticide and not from inactivation of the fungicides. However, where lindane was used alone on seed there was no evidence of germination being lower than that of untreated seed. Severe fungal infection may have masked lindane injury in this latter comparison. None of the insecticides when used alone, showed a consistent value as a fungicide under the severe conditions of this testing procedure.

Table 1.—Influence of insecticides on the action of fungicides used on damaged corn seed in laboratory tests.

Insecticide 1 Oz./Bu.	Germination Rating (100 Max.)[a]			
	No Fungicide[b]	Captan	Dichlone	Thiram
Dieldrin	31	53	83	62
Heptachlor	31	48	86	66
Lindane	25	44	49	46
Aldrin	28	53	81	66
None	24	47	77	60
L.S.D. at 5% level	n.s.	15	13	9

[a] Fungicides were each applied at 0.75 ounce per bushel but in entirely separate tests and no attempt should be made to compare results between fungicides.
[b] Average of three tests.

Insecticides caused no inhibition of fungicides when mixtures were used on injured seed in the field test. Seed treated with the insecticide-fungicide combinations did not produce stands or yields significantly different from the stand and yield of fungicide-treated corn seed (tables 2 and 3). The stands and yields resulting from seed having a fungicide treatment were, however, significantly better than the stand and yield of plants grown from completely untreated seed.

Three levels of dieldrin (table 4) in combination with the three fungicides failed to show any inhibitory effect on the fungicides. In the absence of a fungicide, three dosages of dieldrin produced significantly better stands than completely untreated seed. The yield from untreated seed was less than that from seed treated with dieldrin alone, but in this case the difference was not significant. This response might have been the result

Table 2.—Influence of insecticides on the action of fungicides[a] used on damaged corn seed in field test (stand count).

Insecticide 1 Oz./Bu.	Total Plants per Plot			
	No Fungicide	Captan	Dichlone	Thiram
Dieldrin	28.0	31.3	27.3	32.0
Heptachlor	24.3	28.3	30.3	31.3
Lindane	27.6	30.0	30.0	30.6
Aldrin	24.3	33.6	28.0	32.0
None	24.3	31.0	30.0	30.3
L.S.D. at 5% level	4.7			

[a] Each applied at 0.75 ounce per bushel.

Table 3.—Influence of insecticides on the action of fungicides[a] used on damaged corn seed in field test (yields).

Insecticide 1 Oz./Bu.	Bushels per Acre			
	No Fungicide	Captan	Dichlone	Thiram
Dieldrin	70.3	76.0	73.0	73.3
Heptachlor	57.7	76.0	66.6	75.6
Lindane	70.5	73.7	66.8	66.0
Aldrin	57.2	71.9	73.4	79.3
None	63.2[b]	71.9	66.2	76.6
L.S.D. at 5% level	n.s.			

[a] Each applied at 0.75 ounce per bushel.
[b] No insecticide-no fungicide treatment found to be significantly different from the average of the three fungicide-only treatments.

Table 4.—Influence of dieldrin on the action of fungicides used on damaged corn seed in field test (stand and yield).

Ounces of Dieldrin/Bu.	Total Plants per Plot and Bushels per Acre			
	No Fungicide	Captan	Dichlone	Thiram
0.25	28.6 (67.7)[a]	31.0 (78.5)	28.0 (68.9)	29.3 (79.1)
1	28.0 (70.3)	31.3 (76.0)	27.3 (73.0)	32.0 (73.3)
2	31.0 (72.3)	31.3 (76.7)	31.3 (76.7)	28.6 (71.6)
None	24.3[b] (63.2)[c]	31.0 (71.9)	30.0 (66.2)	30.3 (76.6)
L.S.D. at 5% level		(n.s.)		

[a] Figures in parenthesis indicate bushels per acre.
[b] No dieldrin-no fungicide treatment found to be significantly different from the average of the three dieldrin treatments in absence of fungicide and from the average of the 12 treatments involving fungicides.
[c] No dieldrin-no fungicide treatment not significantly different from the average of the three dieldrin treatments in absence of fungicide nor from the average of the 12 treatments involving fungicides.

of protection from insects, a low order of fungicidal protection from dieldrin or possibly a direct stimulatory effect by dieldrin. An appraisal of dieldrin vs. no-dieldrin in the presence of a fungicide, however, would tend to rule out insect control and stimulation arguments in favor of slight fungicidal action on the part of dieldrin.

Captan, dichlone and thiram had no detrimental effects upon the insecticidal value of dieldrin (table 5).

Table 5.—Bioassay with pomace flies of the influence of fungicides on the insecticidal activity of dieldrin.

Material	LD_{50} P.p.m. Dieldrin	Regression Coefficient
Captan+dieldrin	2.1 (1.5–2.9)	2.1 (1.1–4.0)
Dieldrin	1.8 (1.3–2.6)	2.3 (1.0–5.2)
Dichlone+dieldrin	1.3 (1.0–1.7)	2.5 (2.0–2.9)
Dieldrin	1.3 (1.0–1.8)	2.0 (1.7–2.4)
Thiram+dieldrin	1.2 (0.8–1.8)	2.6 (1.5–4.4)
Dieldrin	1.3 (1.0–1.7)	2.0 (1.7–2.4)

The LD_{50}s of dieldrin used alone (1.3 to 1.8 p.p.m.) were not significantly different from the LD_{50}s of the fungicide-dieldrin mixtures (1.2 to 2.1 p.p.m.). The fungicides themselves were not toxic to the flies when used at a concentration of 4 p.p.m. The regression coefficients were similar, indicating that dieldrin in the mixtures was affecting the insect in the same manner as if used alone.

Summary.—Laboratory tests demonstrated that 1 ounce per bushel of dieldrin, heptachlor or aldrin had no adverse effect on the fungicidal action of normal dosages of captan, dichlone or thiram when used as a combination slurry treatment on injured corn seed. Corn seed treated with lindane in combination with captan showed similar results but when combined with dichlone and thiram there was a significant reduction in germination. Stand count and yield results from a field test involving injured corn seed failed to reveal any adverse effects of the insecticides on the fungicides. Dieldrin used alone showed a low order of fungicidal protection. Bioassays with pomace flies showed that captan, dichlone and thiram had no adverse effects upon the insecticidal action of dieldrin.

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Evaluation of Repellents Against Mosquitoes and Deer Flies in Oregon[1]

I. H. GILBERT,[2] *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

Field evaluations of repellents against the various species of mosquitoes and deer flies in Oregon were made in June and July 1955 in continuation of the research program of the Orlando, Fla., laboratory. The repellents were selected from materials previously tested in the laboratory against *Aedes aegypti* (L.) and in the field against the salt-marsh mosquitoes *Aedes sollicitans* (Wlkr.) and *A. taeniorhynchus* (Wied.). Gilbert *et al.* (1955) reported some of the individual repellents and mixtures to be more effective than the standard repellent (M-250) against the above species and against *Anopheles quadrimaculatus* Say and *Psorophora confinnis* (L.-Arr.). Similar studies were conducted in Alaska by Altman & Smith (1955) and in Panama by Gilbert & Gouck (1955).

The tests against mosquitoes breeding in irrigation water and deer flies, *Chrysops discalis* Will., were conducted at Summer Lake. The tests against mosquitoes breeding in water from melted snow were run in two locations in the Cascade Mountains, Windigo Pass and Diamond Lake. The relative abundance of various species of mosquitoes,[3] as shown by representative collections made while testing, was as follows:

LOCATION	SPECIES	NUMBER COLLECTED
Diamond Lake	*Aedes*	
	fitchii (F. & Y.)	14
	cinereus Meig.	11
	spp. (dark-legged), probably mostly *communis* (Deg.)	82
Windigo Pass	*fitchii*	27
	spp. (dark-legged)	148
Summer Lake	*flavescens* (Muell.)	6
	melanimon Dyar	10
	dorsalis (Meig.)	38

MATERIALS.—The names of the individual repellents and the formulas of the mixtures are given below. Code numbers for mixtures are included for convenient reference.

TESTS WITH REPELLENTS APPLIED TO THE SKIN.—The repellents were used as 50% ethanol solutions against irrigation-water mosquitoes and as 25% solutions against snow-water mosquitoes and deer flies. The repellents were tested by applying 1 ml. of solution, or an equal amount of actual repellent from the pressurized sprays, to the forearm from the wrist to the elbow, and exposing the arm to natural infestations of insects. Against mosquitoes, arms were exposed until a confirmed bite (a bite followed by another within a half hour) was received. The protection time of the repellents against deer flies was based on lands (5 or more seconds duration). The experimental design employed was a round-robin series, i.e., each repellent was paired against each other repellent. An adjusted average protection period, which adjusts for individual variations between host and testing conditions, was computed by the following formula:

$$\text{Average } Ti = \frac{2Ti - Bi}{rn} + M$$

where Ti is the total protection time for all tests with repellent i, Bi is total protection time for both repellents

	INDIVIDUAL CHEMICALS	APPLIED TO SKIN (S) OR CLOTHING (C)
	2-Butyl-2-ethyl-1,3-propanediol	C
	m-Chloro-*N,N*-diethylbenzamide	S
	o-Chloro-*N,N*-diethylbenzamide	S, C
	Diethyltoluamide (technical mixture containing about 72% *meta* isomer)	S, C
	N,N-Diethyl-*m*-toluamide	S
	N,N-Diethyl-*o*-toluamide	S
	N,N-Diethyl-*p*-toluamide	S
	Dimethyl carbate	S
	Dimethyl phthalate	S
	o-Ethoxy-*N,N*-diethylbenzamide	S, C
	Ethyl hexanediol	S
	4-*p*-Methoxyphenyl-2-butanol	S, C
	Propionic acid, ester with *N*-butyl-*N*-(2-hydroxyethyl) propionamide	C
	N,N,o-Triethylbenzamide	S
	MIXTURES	
M-1960	*N*-Butylacetanilide (30%), benzyl benzoate (30%), 2-butyl-2-ethyl-1,3-propanediol (30%), Tween 80 (10%)	C
2020	Dimethyl phthalate (40%), ethyl hexanediol (30%), dimethyl carbate (30%)	S, C
2063	Ethyl hexanediol (50%), *o*-ethoxy-*N,N*-diethylbenzamide (50%)	S, C
2072	Dimethyl phthalate (40%), ethyl hexanediol (30%), *o*-ethoxy-*N,N*-diethylbenzamide (30%)	S, C
2084	*o*-Ethoxy-*N,N*-diethylbenzamide (50%), diethyltoluamide (50%)	S, C
2085	Ethyl hexanediol (33.3%), *o*-ethoxy-*N,N*-diethylbenzamide (33.3%), diethyltoluamide (33.3%)	S, C
2087	Benzyl benzoate (30%), *o*-ethoxy-*N,N*-diethylbenzamide (30%), diethyltoluamide (30%), Tween 80 (10%)	C
2089	Diethyltoluamide (80%), acetate of *o*-phenylphenol (20%)	S
2089E	M-2089 (90%), Tween 80 (10%)	C
2091	Benzyl benzoate (18%), diethyltoluamide (72%), Tween 80 (10%)	C
2092	Ethyl hexanediol (50%), diethyltoluamide (50%)	S
2097	Benzyl benzoate (30%), *N*-butylacetanilide (30%), diethyltoluamide (30%), Tween 80 (10%)	C
2098	*N*-Butylacetanilide (30%), acetate of *o*-phenylphenol (30%), diethyltoluamide (30%), Tween 80 (10%)	C
M-2099	*N*-Butylacetanilide (30%), 1-butyryl-1,2,3,4-tetrahydroquinoline (30%), diethyltoluamide (30%), Tween 80 (10%)	C
2104	Acetate of *o*-phenylphenol (30%), 1-butyryl-1,2,3,4-tetrahydroquinoline (30%), diethyltoluamide (30%), Tween 80 (10%)	C
2105	Lindane (3%), diethyltoluamide (97%)	C
2107	1-butyryl-1,2,3,4-tetrahydroquinoline (35%), diethyltoluamide (55%), Tween 80 (10%)	C
	PRESSURIZED SPRAYS	
OM-855	Diethyltoluamide (25%), Freon 11 and 12 (75%)	S, C
859	Diethyltoluamide (30%), Freon 11 and 12 (40%), isopropanol (30%)	S, C

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[3] Determinations were made by Dr. Alan Stone of the Entomology Research Branch, U. S. Department of Agriculture.

Table 1.—Comparative effectiveness of individual repellents and M-2020 as skin applications against *Aedes dorsalis* and *Chrysops discalis* at Summer Lake, Oreg.[a]

Repellent	Average Protection Time (Minutes) Against—		Ratio to M-2020	
	dorsalis	*discalis*	*dorsalis*	*discalis*
First Series				
M-2020	224	108	1.00	1.00
Ethyl hexanediol	304	106	1.35	.98
o-Chloro-*N,N*-diethylbenzamide	288	97	1.29	.90
o-Ethoxy-*N,N*-diethylbenzamide	344	99	1.54	.92
Diethyltoluamide	404	119	1.80	1.10
Second Series				
M-2020	221	116	1.00	1.00
Dimethyl phthalate	162	107	.73	.92
Dimethyl carbate	171	133	.77	1.15
N,N,o-Triethylbenzamide	375	114	1.69	.98
m-Chloro-*N,N*-diethylbenzamide	391	114	1.77	.98
Third Series				
M-2020	231	—	1.00	—
4-*p*-Methoxyphenyl-2-butanol	144	—	.62	—
N,N-Diethyl-*o*-toluamide	335	—	1.45	—
N,N-Diethyl-*m*-toluamide	336	—	1.45	—
N,N-Diethyl-*p*-toluamide	193	—	.84	—

[a] Average of 4 tests, with 50% ethanol solutions against *dorsalis* and 25% against *discalis*.

in all pairs in which repellent i occurred, n is the total number of repellents, r is the number of times each pair was replicated, and M is the grand mean of all protection times in the series.

Individual repellents.—Twelve individual repellents were compared with the standard, M-2020, in three series of tests at Summer Lake (table 1) and the first two series were repeated in the Cascade Mountains (table 2).

In tests against *Chrysops discalis* none of the repellents differed very markedly from M-2020 in effectiveness. Dimethyl carbate and diethyltoluamide had slightly higher ratios of effectiveness than the standard, whereas dimethyl phthalate and two *N,N*-diethylbenzamides (*o*-chloro- and *o*-ethoxy-) had slightly lower ratios.

Against all species of mosquitoes diethyltoluamide was the most effective repellent, with protection times about twice as long as those with M-2020. The *o*-chloro- and *o*-ethoxy-analogs of *N,N*-diethylbenzamide were about equal in effectiveness to the standard against mosquitoes at Diamond Lake and 30 to 50% more effective against the mosquitoes at Summer Lake and Windigo Pass. Ethyl hexanediol was more effective than M-2020 against *Aedes dorsalis* but less effective against the mosquitoes breeding in snow water. The other two ingredients of M-2020, dimethyl phthalate and dimethyl carbate, were about equal to or less effective than the mixture. The *N,N,o*-triethyl- and the *m*-chloro-*N,N*-diethylbenzamides were from 60 to 90% more effective than M-2020 against mosquitoes at all test locations.

Against *Aedes dorsalis* the *ortho* and *meta* isomers of *N,N*-diethyltoluamide were 45% more effective than M-2020, whereas the *para* isomer was 16% less effective. The remaining compound, 4-*p*-methoxyphenyl-2-butanol, was the least effective of the repellents tested.

Repellent mixtures.—A round-robin series of tests was conducted to compare the effectiveness of seven repellent mixtures, including the standard, M-2020. The results of the tests conducted at Summer Lake with mosquitoes and deer flies are presented in table 3, and those against snow-water mosquitoes in the Cascade Mountains in table 4.

Against *discalis*, M-2092, which contained equal parts of diethyltoluamide and ethyl hexanediol, was slightly more effective than the other mixtures, which were about equal to M-2020. All the mixtures were more effective than M-2020 against all species of mosquitoes, but the differences were not as great against *dorsalis* as against the snow-water *Aedes*. The mixtures that contained

Table 3.—Results of tests with seven repellent mixtures as skin applications against *Aedes dorsalis* and *Chrysops discalis* at Summer Lake.[a]

Repellent	Average Protection Time (Minutes) Against—		Ratio to M-2020	
	dorsalis	*discalis*	*dorsalis*	*discalis*
M-2020	236	156	1.00	1.00
2063	266	155	1.13	.99
2072	270	164	1.14	1.05
2084	328	166	1.39	1.06
2085	340	157	1.44	1.01
2089	362	166	1.53	1.06
2092	373	188	1.58	1.20

[a] Average of six tests with 50% ethanol solutions against *dorsalis* and 25% against *discalis*.

Table 2.—Relative effectiveness of eight individual repellents and M-2020 as skin applications against snow-water mosquitoes at Windigo Pass and Diamond Lake.[a]

Repellent	Average Protection Time (Minutes) at—		Ratio to M-2020 at—	
	Windigo Pass	Diamond Lake	Windigo Pass	Diamond Lake
First Series				
M-2020	233	157	1.00	1.00
Ethyl hexanediol	157	135	.67	.86
o-Chloro-*N,N*-diethylbenzamide	346	165	1.48	1.05
o-Ethoxy-*N,N*-diethylbenzamide	360	149	1.55	.95
Diethyltoluamide	447	325	1.92	2.07
Second Series				
M-2020	195	127	1.00	1.00
Dimethyl phthalate	176	150	.90	1.19
Dimethyl carbate	172	125	.88	.98
N,N,o-Triethylbenzamide	376	245	1.88	1.92
m-Chloro-*N,N*-diethylbenzamide	314	225	1.61	1.77

[a] Average of 4 tests with 25% ethanol solutions.

Table 4.—Relative effectiveness of seven repellent mixtures as skin applications against snow-water mosquitoes at Windigo Pass and Diamond Lake.[a]

Repellent	Average Protection Time (Minutes) at—		Ratio to M-2020 at—	
	Windigo Pass	Diamond Lake	Windigo Pass	Diamond Lake
M-2020	210	141	1.00	1.00
2063	304	192	1.45	1.36
2072	330	170	1.57	1.21
2084	339	241	1.61	1.71
2085	347	227	1.65	1.61
2089	366	280	1.74	1.99
2092	407	265	1.94	1.88

[a] Average of six tests with 25% ethanol solutions.

Table 5.—Protection times with diethyltoluamide in pressurized sprays and as a 50% ethanol solution against *Aedes dorsalis*.[a]

Formulation	Grams Applied	Average Protection Time (Minutes)	Ratio to Diethyltoluamide
Solution	1.0	373	1.00
OM-855	2.0	365	.93
OM-859	1.7	351	.89

[a] Average of 9 tests.

diethyltoluamide were more effective than those that did not contain it, and none of the mixtures showed a higher ratio to M-2020 than diethyltoluamide alone (tables 1 and 2).

Diethyltoluamide in pressurized sprays.—A series of tests was conducted against *dorsalis* with diethyltoluamide, applied to the arms as an ethanol solution and as an aerosol or a pressurized spray using Freon as the propellent. All formulations were applied at 0.5 gram of diethyltoluamide per forearm, and the arms were rubbed with the hand to insure complete coverage.

The results of the tests, given in table 5, indicate that the aerosols and pressurized sprays were very slightly less effective than the ethanol solution.

Tests with Repellents Applied to Clothing.—Six individual repellents, 16 mixtures, and 2 pressurized spray formulations were tested as treatments for application to clothing.

Tests with stockings.—Acetone solutions of the repellents were applied to cotton stockings. The dosages per square foot were 2 grams of the individual repellents, 2.3 grams of M-2105, 2.8 grams of M-2089E and M-2091, and 3.6 grams of the other mixtures. Tests were made after the stockings had aged for 1, 6, and 26 days, and after 1½-minute rinses following the aging period. During the aging period the stockings hung on lines inside a closed building. Rinsing was accomplished by agitating the stockings singly in 3 pints of water. At each test each stocking was exposed for 5 minuts on each of three hosts, or until an average of at least three bites per host was obtained, and at least two hosts had one or more bites.

Diethyltoluamide, 2-butyl-2-ethyl-1,3-propanediol, *o*-chloro-*N*,*N*-diethylbenzamide, *o*-ethoxy-*N*,*N*-diethylbenzamide, and all the mixtures were highly effective after 26 days of aging, allowing an average of 0 to 0.5 bites. 4-*p*-Methoxyphenyl-2-butanol and ester of propionic acid with *N*-butyl-*N*-(2-hydroxyethyl)propionamide allowed an average of more than one bite after 26 days of aging. After a half-minute rinse, stockings treated with M-2097, M-2098, M-2099, M-2104, M-2106, M-2107, 2-butyl-2-ethyl-1,3-propanediol, and *o*-chloro-*N*,*N*-diethylbenzamide allowed an average of less than one bite. The other repellents and mixtures were less effective. All were ineffective, allowing an average of more than five bites, after the second rinse. After rinsing, diethyltoluamide was slightly more effective applied as an acetone solution than as pressurized sprays.

Tests with shirts.—Tests were conducted with diethyltoluamide applied to shirts as an acetone solution and as pressurized sprays. Each treatment was applied to three shirts. M-1960 was included in the tests as a standard. After 26 days of aging the shirts gave complete protection, and were rinsed by machine in cold water for 5 minutes and retested. Shirts treated with the acetone solution of diethyltoluamide were slightly more effective than those treated with pressurized sprays, but none provided satisfactory protection. M-1960 allowed less than two bites after one rinse but afforded no protection after the second rinse.

Summary.—Field studies with repellents were conducted in Oregon in the summer of 1955 against mixed populations of mosquitoes breeding in snow water, principally *Aedes communis* (Deg.); against mixed populations of mosquitoes breeding in irrigation water, principally *Aedes dorsalis* (Meig.); and against deer flies, *Chrysops discalis* Will.

As skin applications against all species of mosquitoes, diethyltoluamide was the most effective of the 12 individual repellents tested. This repellent was about twice as effective as the standard, M-2020, (containing dimethyl phthalate (40%), dimethyl carbate (30%), and ethyl hexanediol (30%)). Against *A. dorsalis* the *ortho* and *meta* isomers of *N*,*N*-diethyltoluamide were 45% more effective than M-2020, whereas the *para* isomer was 16% less effective.

All the new mixtures caused longer protection time than M-2020 against all species of mosquitoes. These differences were not as pronounced against *dorsalis* as against the snow-water mosquitoes. The mixtures that contained diethyltoluamide were more effective than those that did not. Against *Chrysops discalis* none of the repellents differed very markedly from M-2020 in repellency.

Ethanol solutions or pressurized sprays of diethyltoluamide were about equal in effectiveness as skin applications.

The 16 mixtures, 2 pressurized spray formulations, and 4 of the 7 individual repellents tested as clothing treatments showed almost complete protection from mosquitoes after 26 days of aging. All were ineffective after two rinses.

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Susceptibility of *Macrocentrus ancylivorus* and Other Parasites of the Oriental Fruit Moth to Insecticides[1]

H. W. Allen, *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*[2]

In the eastern half of the United States for at least 20 years before DDT was used against the oriental fruit moth (*Grapholitha molesta* (Busck)), it was very heavily attacked by insect parasites. When parasitism of twig-infesting larvae was high, fruit injury was low (Yetter & Allen 1940, Allen 1943). As soon as DDT and BHC began to be used in orchard spraying, many entomologists and growers thought that parasites in peach orchards would be decimated, and were concerned at the effect on control of the pest. The older insecticides, which killed by hitting or being ingested by the insect, caused no appreciable mortality among the fruit moth parasites. These new insecticides killed most of the insects that even walked for short distances over dry spray residues.

Peterson (1946) reported that when five species of parasites were exposed to dry deposits of DDT on peach leaves that had been applied at a dilution of 1:10,000, there was 100% mortality of all species within 40 hours. DDT was more toxic to *Macrocentrus ancylivorus* Roh. than to the two species of *Cremastus* tested. High mortalities of *ancylivorus* occurred at dilutions as low as 1:100,000, and also when they were exposed to foliage 58 days after it had been sprayed with a 1:1,000 dilution. Peterson also found that, among the hymenoptera tested, males and females were equally susceptible to DDT, but Pielou & Glasser (1951), exposing *ancylivorus* for a few minutes to dry films of crystalline DDT in celloidin, found males to be more susceptible. Fleming *et al.* (1951), using dilute suspensions of insecticides on moist filter paper, found that *ancylivorus* reacted differently to DDT than to chlordane, and that males reacted faster than females only when the sexes were exposed separately.

This paper discusses laboratory experiments at Moorestown, N. J., undertaken to determine the relative susceptibility of the oriental fruit moth and some of its parasites to spray deposits of DDT and parathion.

Tests of Weathered Deposits of DDT on Absorbent Paper.—In one experiment undertaken in 1944 sheets of paper toweling were sprayed with a suspension of DDT, and then exposed in wooden racks to the weathering effects of sunlight and rain. A 50% concentration in pyrophyllite was micronized and used at the rate of 2 pounds to 100 gallons, to which 1 pint of glue and 1 quart of summer oil were added. After weathering, dry sheets were transferred to battery jars for testing. Each sheet was inserted vertically as a lining to the jar with the sprayed surface facing inward. Each jar was provided with fresh food and water. Ten parasites and 10 moths were exposed in each jar, and the dead were counted after 48 hours. The results with papers that had been weathered for different periods are shown in table 1. The deposits lost much of their toxicity to moths in less than 3 weeks, but continued to be highly toxic to the parasites for 5 weeks.

Tests with DDT on Caged Trees.—In 1944 also the effect of DDT on parasitism was studied on several peach trees enclosed in cloth-covered cages. In an experiment conducted during the first brood of the fruit moth, two young nonbearing trees were sprayed on May 10 with DDT and summer oil of the formula described above, and two trees serving as checks received summer oil without DDT. A cage was placed over each tree, and on May 11 each was stocked with 5 female and 5 male oriental fruit moths, and with 75 female and 25 male *ancylivorus* parasites. Five days later no living parasites could be found in the cages over the DDT-sprayed trees, but 67 out of the original 200 were found alive in the cages over the check trees. Living moths were found in the cages over both check and DDT-sprayed trees. On May 24, thirteen days after the moths and parasites were introduced, the infested twigs on the caged trees were collected and the moths and parasites were reared. Since duplicate cages gave similar results, the data are the summation of the duplicates.

Table 1.—Per cent mortality of oriental fruit moths and *Macrocentrus ancylivorus* after 48 hours' exposure to dry paper containing weathered residues of DDT.

Days Exposed to Weathering	Parasites		Fruit Moths	
	Sprayed	Check	Sprayed	Check
1	100	0	100	10
19	90	30	20	10
34	100	20	0	0
50	10	10	0	0
65	20	0	10	0

Although the cages obviously were overstocked with parasites, the data are interesting. In the DDT-sprayed trees 117 mature oriental fruit moth larvae were reared, from which 110 moths emerged and 6% were parasitized by *ancylivorus*. In the check trees only 17 oriental fruit moth larvae were reared, from which 2 moths emerged and 88% were parasitized. The large difference in parasitization between the DDT-sprayed and the check trees was foreshadowed by the difference in mortalities among the parasites introduced. However, the relatively large number of oriental fruit moth larvae reared from the DDT-sprayed trees was unexpected. It was about seven times as large as from the check trees. Apparently the insecticide checked the parasites but failed to check moth propagation. The small number of larvae reared from the checks was also unexpected, and was probably due to superparasitism killing larvae before they had matured. The moths emerging from the DDT-sprayed trees were 55 times as numerous as from the check trees.

When the cages were examined for *ancylivorus* adults 13 days from the first introduction and none were found, a second lot of 25 females and 25 males was released in

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[2] The experiments reported were conducted by the late W. P. Yetter, Jr., and the author. R. D. Chisholm and Louis Koblitsky provided the spray formulations and made helpful suggestions.

each cage, or a total of 100 in the duplicates. Five days later 34 living parasites were counted in the cages over DDT-sprayed trees, and 55 in the checks. Since the average life span of *ancylivorus* does not greatly exceed 5 days, the rate of survival on the DDT-sprayed trees was good, but less than on the check trees. When infested twigs were collected again on June 1, 20 days after spraying and 7 after the second release of parasites, there was 100% parasitization in all the trees.

In this experiment DDT used at the customary rate (1 pound per 100 gallons) did not inhibit propagation of the oriental fruit moth, but at first greatly reduced parasitization by *ancylivorus*. However, in 2 to 3 weeks mortalities of adult parasites were only moderate and parasite propagation was again up to a high level.

A similar experiment was conducted against the second brood of moths. Twelve young peach trees were sprayed on June 16, six with DDT-oil and six with oil only. These trees were used in a series of three tests, in each of which cages were placed over two DDT-sprayed trees and two of the oil-sprayed checks, and 5 oriental fruit moths and 25 *ancylivorus* of each sex were introduced into each cage. The day after spraying, all naturally infested twigs were removed from four trees, and cages were placed over them for the first test of the series. In this test moths and parasites were introduced 1 day after spraying. In a second test the moths were released in each cage after 15 days and the parasites after 19 days, and in a third test, respectively, after 30 and 34 days. Counts of parasites found alive in the cages were made each day for 5 days. Where the living parasites were numerous, some were missed in each count, and on some days more living were counted than on the previous day. In each cage two collections of infested twigs were made 4 to 6 days apart, and the progeny were reared. The results are presented in table 2. Since each experiment and check were duplicated with small differences between pairs, the results for each pair have been totaled and thus each line in the table presents results from the introduction of 20 moths and 100 parasites.

Much of the exposure during these tests was in hot midsummer weather, and the high-temperature extremes in the cages were higher than in the orchard outside. This presumably shortened the life span of the parasites and perhaps also adversely affected the moths. Toward the end of the experiment hardening of the twigs greatly reduced the rate of moth propagation.

When the moths and parasites were released 1 day after the spraying, no living parasites could be found on the sprayed trees after 2 days but more than half the original number were present in the checks. DDT did not retard the propagation of oriental fruit moth larvae in these cages, the increase being 12-fold compared to 10-fold in the check. Among parasites introduced 19 or 34 days after spraying, there were fewer living parasites on the sprayed trees than on their checks at each of the daily counts, but after the large initial reduction in the sprayed trees the mortality among the survivors tended to level off. In the first series there was no propagation of parasites on the sprayed trees, but in the other two series it was somewhat higher in the sprayed trees than in their respective checks.

Table 2.—Effect of exposing second-brood oriental fruit moths and *Macrocentrus ancylivorus* on caged trees after spraying with DDT.

Parasites Introduced After Spraying	Number of Live Parasites After (Days)					Oriental Fruit Moths		
	1	2	3	4	5	Larvae Reared	Moths Emerged	Per Cent Parasitized
1 day:								
Sprayed	1	0	—	—	—	248	246	0
Check	51	65	—	—	—	200	26	87
19 days:								
Sprayed	32	23	15	17	15	28	4	83
Check	43	36	38	44	29	19	4	75
34 days:								
Sprayed	38	21	13	5	—	11	5	44
Check	55	37	33	14	—	50	35	29

The data indicate that DDT spray on peach foliage is much more toxic to adult *ancylivorus* than to oriental fruit moths, and that although some of the parasites are killed up to 34 days after spraying, satisfactory parasitism may occur as early as 19 days after.

TESTS WITH RESIDUES OF DDT AND PARATHION ON SPRAYED APPLES.—In 1953 and 1954 many laboratory tests were made to ascertain the toxicity to the oriental fruit moth and several of its parasites of residues of DDT and parathion on apple. Immature apples about 1 inch in diameter were used, and adult insects were exposed to dry residues on the skin of the apples. Whatever parasites were available when the tests were set up were used. They included *Macrocentrus ancylivorus*, *Horogenes molestae* (Uch.), *Phanerotoma grapholithae* Mues., and *Agathis festiva* Mues. In most of the tests the toxicity was determined after 48 hours of exposure. Individuals unable to fly and displaying erratic activity were considered moribund. Freshly emerged insects were used, and the insects in each test unit were selected from the same stock of adults. Sweetened food was always placed out of contact with sprayed surfaces of the apples.

In preliminary tests made in March 1953, unsprayed apples were dipped in a suspension containing DDT at the rate of 1 pound to 100 gallons of water. One apple was placed in a jelly glass, and 10 parasites were introduced into each glass. There was one glass containing an unsprayed apple for each glass containing an apple dipped in DDT. The survival was 12% in the *Horogenes molestae* exposed to DDT as compared with 95% in the checks (average of 4); 60% in *Phanerotoma grapholithae* compared with 100% in its check (1 test); and 90% in *Agathis festiva* compared with 100% in its check (1 test). Thus there was some survival of *molestae*, only moderate toxicity among the *grapholithae*, and almost none among the *festiva*, even when exposed to rather heavy deposits of DDT.

In recent years it has been necessary to use, for our mass rearing of parasites, apples thinned in late June from orchards sprayed by the owners with DDT or parathion. A study of the means of removing spray residues has provided some information on the tolerance of the parasites to these residues. In 1953 sprayed apples were obtained from two orchards, in one of which DDT and in the other parathion had been used a few days before. No other insecticide except lead arsenate had preceded the DDT spraying in the first orchard, but DDT had been used in the second orchard. Analyses of the residues on the latter showed that parathion was present when the apples were stored, and that it persisted for many weeks.

Table 3.—Survival of the oriental fruit moth and its parasites exposed to insecticides on immature apples obtained from sprayed orchards.

Insect	Bearing Spray Residues		Checks	
	Number of Lots	Per Cent Survival	Number of Lots	Per Cent Survival
Apples sprayed with DDT				
Macrocentrus ancylivorus	3	14	1	85
Horogenes molestae	14	33	9	86
Phanerotoma grapholithae	4	92	2	95
Agathis festiva	5	78	4	96
Oriental fruit moths	7	87	2	100
Apples sprayed with parathion				
Horogenes molestae	5	12	5	9
Phanerotoma grapholithae	2	0	2	1006
Agathis festiva	2	90	2	90
Oriental fruit moths	1	0	1	100

In the first few weeks after these apples were picked and stored, several series of tests were made with parasites and moths exposed to the apples under different conditions. In some tests jelly glasses and in others covered wooden trays were used as the units of exposure. Fair uniformity in the microclimate was obtained by using measured quantities of apple, and sand and water to provide a moist floor. In both types of cage the residues remained dry, but the humidity was relatively high. Temperatures in an air-conditioned walk-in cage housing the test lots were maintained at 80°–85° F. In each series there were check lots of unsprayed apples. Ten or 20 insects, half of each sex, were used in the exposure. The insects were exposed to the insecticides for 48 hours. The results are shown in table 3. Each value is the average of all the tests in the series.

The DDT residues were highly toxic to *Macrocentrus ancylivorus* and *Horogenes molestae*, but only slightly so to *Phanerotoma grapholithae*, *Agathis festiva*, and oriental fruit moths. Small parathion residues were very toxic to *molestae*, *grapholithae*, and oriental fruit moths, but did not show any toxicity to *festiva* in two small tests.

In the late winter of 1953–4, several months after the apples with parathion residues had been placed in cold storage, it had been found feasible to reduce the residue enough to eliminate losses among breeding parasites used in the mass production of *Agathis festiva* and *Phanerotoma grapholithae*. When propagation rates dropped below customary levels, the situation was investigated. It was suspected that when apples from the parathion-sprayed orchard were used in the egg-deposition cages the mortality among the oriental fruit moths was unduly high, even though no unusual mortality occurred among the breeding parasites used on the same apples.

To show the pattern of survival, moths were exposed to apples on platforms in jelly glasses over moist sand at 80° F. Apples from the parathion-sprayed stock were compared with those from DDT-sprayed stock. All samples were given a routine wash in benzene-isopropyl alcohol mixture to reduce the residues. Ten newly emerged moths (5 of each sex) were placed in each cage, and observations on survival were made daily. On the parathion-sprayed stock the only mortality for the first 3 days was 10% in one cage, but survival dropped to 50–90% after 4 days and to 20–40% after 5 days. The only mortality in the DDT-sprayed check stock was 10% after 5 days.

Usually insects tolerating exposure to chlorine and phosphorus insecticides for 48 hours without signs of injury are not harmed by longer exposures. However, in this experiment there was no evidence of toxicity even after 72 hours, but there was moderate mortality after 96 hours, and heavy mortality after 120 hours. This delayed mortality was probably due to traces of parathion not removed by washing which were so minute that the parasites exposed were not affected. These preliminary observations suggest that there may be times when the residual effect of parathion may be more potent on an orchard pest than on some of its parasites.

SUMMARY.—Experiments and observations were made to determine whether parasites of the oriental fruit moth (*Grapholitha molesta* (Busck)) are more susceptible than their host to residual insecticides such as DDT and parathion.

When DDT of the usual concentration was sprayed on paper toweling and the paper allowed to weather, the deposits lost much of their toxicity to oriental fruit moths in less than 3 weeks, but remained highly toxic to adults of *Macrocentrus ancylivorus* Roh. for 5 weeks. Similar results were obtained when oriental fruit moths and *ancylivorus* adults were introduced in cages placed over small peach trees sprayed with DDT (1 lb./100 gal.). The insecticide failed to stop infestation by the first two broods of twig-infesting oriental fruit moth larvae, but completely eliminated parasitism for a few days. Some adult parasites on sprayed trees were killed up to 34 days after spraying, but satisfactory parasitism occurred when parasites were exposed 19 days after spraying.

In laboratory tests with immature apples from sprayed orchards, DDT residues were highly toxic to *Macrocentrus ancylivorus* and *Horogenes molestae* (Uch.), but only slightly so to *Phanerotoma grapholithae* Mues., *Agathis festiva* Mues., and oriental fruit moths. Small parathion residues were extremely toxic to *molestae*, *grapholithae*, and oriental fruit moths, but not to *festiva*. Very small residues of parathion not toxic to either *grapholithae* or *festiva* appear to have caused a heavy, delayed mortality of oriental fruit moths, first becoming noticeable after 96 hours' exposure.

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European Corn Borer Control in Relation to Malathion Residues[1]

H. C. Cox,[2] J. E. Fahey,[2] and T. A. Brindley[2,3]

Malathion has been very effective for the control of several insect pests. Ginsburg *et al.* (1952) reported that compound 4049 (malathion) gave some control of the European corn borer (*Pyrausta nubilalis* (Hbn.)) but was not as effective as DDT, parathion, or ryania. Questel & Brindley (1953) reported 55 and 71% reductions in corn borer populations following malathion sprays. Such reports, combined with the fact that malathion has a low mammalian toxicity, suggested further experimentation. In 1954 and 1955 tests were conducted at the European corn borer research laboratory, Ankeny, Iowa, in cooperation with the Iowa Agricultural Experiment Station.

Materials and Procedure.—The malathion was applied both in sprays and granules. The following formulations were used: a 25% wettable powder, a 57% emulsifiable concentrate, and 10% on granular Celite. In 1955 a 25% DDT emulsifiable concentrate and 7.5% DDT on granular attapulgite were included for comparison.

A high-clearance sprayer was used to spray four rows of corn simultaneously. The machine was calibrated to deliver 10 gallons of liquid per acre at a pressure of 40 pounds per square inch. Two fan-type nozzles, approximately 13 inches apart, were mounted on a T-shaped drop over each row. The nozzles were directed down and slightly in toward each row. The reciprocating-chain type seeder described by Lovely *et al.* (1956) was used to apply the granulated materials.

Each plot was four rows wide and 140 feet long. Only the two center rows were used for study. When the first corn borer egg masses were found in the field, 10 plants in each untreated plot were randomly tagged for further observation. Records were taken on the number of new, missing, and hatched egg masses and on the number of plants showing borer damage to the leaves on each Monday, Wednesday, and Friday. A felt-tip fountain pen filled with indelible ink was used to mark the location of the egg masses.

The procedure for dissecting plants reported by Cox *et al.* (1956) was used. Records were taken on the number of borers and borer tunnels in each plant. Since there was a high correlation between borers and tunnels, only data on borers are reported herein.

In 1954 the last application of malathion was made on July 8. During the afternoon of that day 10 corn plants were taken at random from each plot, weighed, and then run through a small silage chopper. A quart ice-cream carton was filled with a sample of the chopped material and the sample weighed and frozen.

In 1955 plants were sampled in the manner described by Fahey *et al.* (1956).

Samples of the frozen corn plants were shipped to the Entomology Research Branch laboratory at Vincennes, Ind., where they were thawed and brought to room temperature. Each was then transferred to a 1-gallon glass jar, 250 ml. of carbon tetrachloride added, and the jar sealed and tumbled for 1 hour. The carbon tetrachloride solution of the residue was filtered into storage bottles.

The analytical technique described by Norris *et al.* (1954) was used without modification. Carbon tetrachloride extracts of untreated corn gave no interference in the analytical method. In 1954 recovery of malathion added to corn stalks varied from 95 to 104%.

Experiment on Field Corn.—In 1954 an experiment on the borer-susceptible double-cross Ia 4297 was designed to compare the following: (1) formulations—wettable powders and emulsifiable concentrates; (2) rates of application—1 and 2 pounds per acre; (3) number of applications—one, two, and three; and (4) dates of application—5-day intervals between June 18 and July 8. The experiment was set up as a randomized complete block with each treatment replicated four times.

Borer Control.—Table 1 is a summary of the results obtained. The wettable powder did a significantly better job of controlling borers than the emulsifiable concentrate. Plots treated with 2 pounds of malathion had significantly fewer borers than those treated with 1 pound.

Table 1.—Number of first-brood European corn borer larvae per 100 field corn plants after treatment with malathion,[a] 1954.

Application Dates	1 lb. per Acre		2 lb. per Acre	
	Emulsion	Wettable-powder Spray	Emulsion	Wettable-powder Spray
June 18	477	501	405	460
23	316	209	200	177
28	475	225	352	245
June 18 and 23	354	385	230	155
23 and 28	354	145	167	88
28 and July 3	327	322	209	261
June 18, 23, and 28	175	172	236	60
23, 28, and July 3	232	149	142	120
28, July 3 and 8	284	166	157	155

[a] Untreated plots, 354 larvae per 100 plants.

Generally two applications of insecticide gave better control than one while three applications were better than two.

The best single date of application was June 23. Plots which showed the best borer control with two and three applications had June 23 and 28 application dates in common. As shown in figure 1, there was an increase

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[2] Entomology Research Branch, Agr. Res. Serv., U.S.D.A.
[3] Iowa State College.

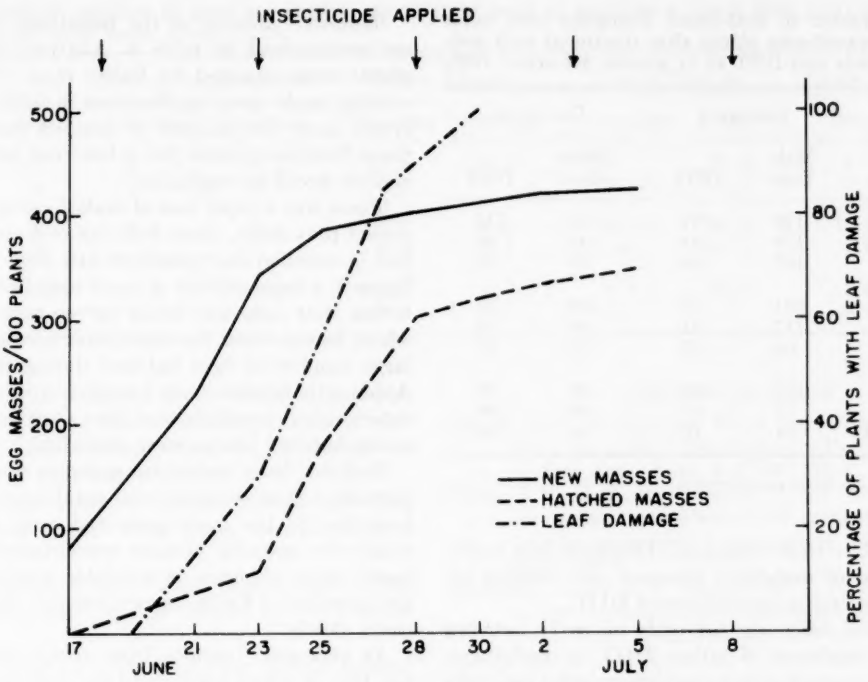

FIG. 1.—Time of insecticide applications to field corn in relation to corn borer egg laying, hatching, and leaf-feeding damage in 1954.

from 30 to 90% in plants showing leaf feeding between these two dates. Egg laying was beginning to drop off sharply on June 23, whereas hatching was starting to increase.

Residues.—Results of the residue analyses are summarized in table 2. Samples from plots which were treated last were analyzed first. When the analyses showed that they contained less than 0.1 p.p.m. of malathion, the samples from plots treated on an earlier date were not analyzed.

Most of the residue was lost in 5 days. Such rapid loss of insecticide agreed with the findings of other workers. Ginsburg *et al.* (1952) noted an absence of residue on sweet corn 21 days after applications of malathion dust. They reported a complete loss in 2 days of a 0.8-pound (74 p.p.m. deposit) application in lima beans and found only 1 p.p.m. 3 days after a 1.6-pound (94.4 p.p.m. deposit) application. Westlake & Butler (1953) reported a malathion residue of only 4 p.p.m. on spinach 6 days after a desposit of 61 p.p.m.

Residues on plants treated with 2 pounds of malathion per acre were much heavier than those on plants treated with 1 pound.

More residue was deposited from the emulsion sprays than from the wettable-powder sprays. In contrast, Smith *et al.* (1954) reported greater deposits from wettable powders than from emulsions on green onions. However, in agreement with their findings, the residue from wettable-powder sprays was more persistent than that from emulsion sprays. The longer persistence of malathion from wettable powders may have been responsible for the better borer control obtained with that formulation.

Nine out of 14 treatments with a reduction in borer populations of 50% or better had in common June 23 and 28 as dates of application (table 1). Between these two dates almost 70% of the hatching occurred (fig. 1). Consequently, it would seem that a sufficient amount of malathion was present on these treatment dates to kill a large percentage of the very young larvae present. Residue loss was so rapid that treatments earlier or later than June 23 and 28 had deteriorated before a large number of larvae were present.

Table 2.—Malathion residues, in parts per million, on field-corn plants after treatment for control of first-brood European corn borer, 1954.

Application Dates	Days from Last Treatment to Sampling on July 8	1 lb. per Acre		2 lb. per Acre	
		Emulsion	Wettable-powder Spray	Emulsion	Wettable-powder Spray
June 28, July 3 and 8	0	18.9	14.3	52.1	40.3
June 28 and July 3	5	0	0.7	0.6	7.0
June 23, 28, and July 3	5	0	0.5	0.4	6.8
June 23 and 28	10	—	—	<0.1	<0.1
June 18, 23, and 28	10	—	0	<0.1	<0.1

EXPERIMENT ON SWEET CORN.—Green Giant Code 10 sweet corn was used in 1955 to compare the following: (1) toxicants—malathion and DDT; (2) formulations—emulsifiable concentrates and granules; (3) number of applications—one, two, and three; and (4) dates of application—5-day intervals between treatments beginning June 2 and ending June 24. DDT was applied at 1½ pounds per acre and malathion at 2 pounds. The experimental design was a split-split plot with each treatment replicated four times.

Borer Control.—Table 3 is a summary of the results obtained. An analysis of variance showed that DDT

Table 3.—Number of first-brood European corn borer larvae per 100 sweet-corn plants after treatment with malathion at 2 pounds and DDT at 1½ pounds per acre,[a] 1955.

Application Dates (June)	Emulsion		Granules	
	Malathion	DDT	Malathion	DDT
2	149	137	171	115
9	179	95	146	92
14	167	62	65	51
2 and 9	201	76	145	65
9 and 14	117	51	92	74
14 and 19	65	25	79	27
2, 9, and 14	114	40	54	62
9, 14, and 19	71	27	43	50
14, 19, and 24	34	16	43	29

[a] Untreated plots, 161 larvae per 100 plants.

was more effective than malathion. However, late multiple applications of malathion granules gave control almost as good as similar applications of DDT.

There was no difference (at odds of 19:1) between granules and emulsions of either DDT or malathion. However, plots treated with malathion granules generally had fewer borers than those treated with emulsions.

The effect of time of application in relation to borer control may be shown by comparing table 3 with figure 2. The late dates of application gave significantly better borer control than the early dates. Four times as many egg masses hatched between June 14 and 19 as prior to June 14. Plots having in common June 19 as a treatment date had good corn borer control with malathion.

Residues.—Results of the malathion residue analyses are summarized in table 4. Analyses of DDT-treated plants were reported by Fahey *et al.* (1956). Plots receiving single spray applications in table 3 were not analyzed, since the analysis of samples from plots having those treatment dates plus a later one indicated that the residue would be negligible.

There was a rapid loss of malathion in 5 days. As discussed previously, plots with the best corn borer control had in common the treatment date June 19. As shown in figure 2, a large number of borer eggs hatched a few days before that date and hence larvae were feeding on the whorl leaves when the malathion was applied. Another large number of eggs hatched during the next 2 days. Apparently residues from treatments prior to June 19 had deteriorated so rapidly that they were not effective when newly-hatched larvae were abundant.

Residues from malathion granules were slightly more persistent than residues from emulsions. This may have been due to the more protected areas of the plant in which the granules became concentrated On the other hand, some evidence is available which indicates that the granulated Celite carrier releases impregnated toxicants slowly.

As expected, residues from sprays were greatest on the tips of whorl leaves and on stalk leaves, and those from granules were greatest in the whorls of the plants and in the stalk and leaf axil area. Plants treated with malathion granules had considerably less residue than plants treated with emulsions.

Summary.—The use of malathion for control of the European corn borer (*Pyrausta nubilalis* (Hbn.)) was studied on field corn in 1954 and on sweet corn in 1955, at Ankeny, Iowa.

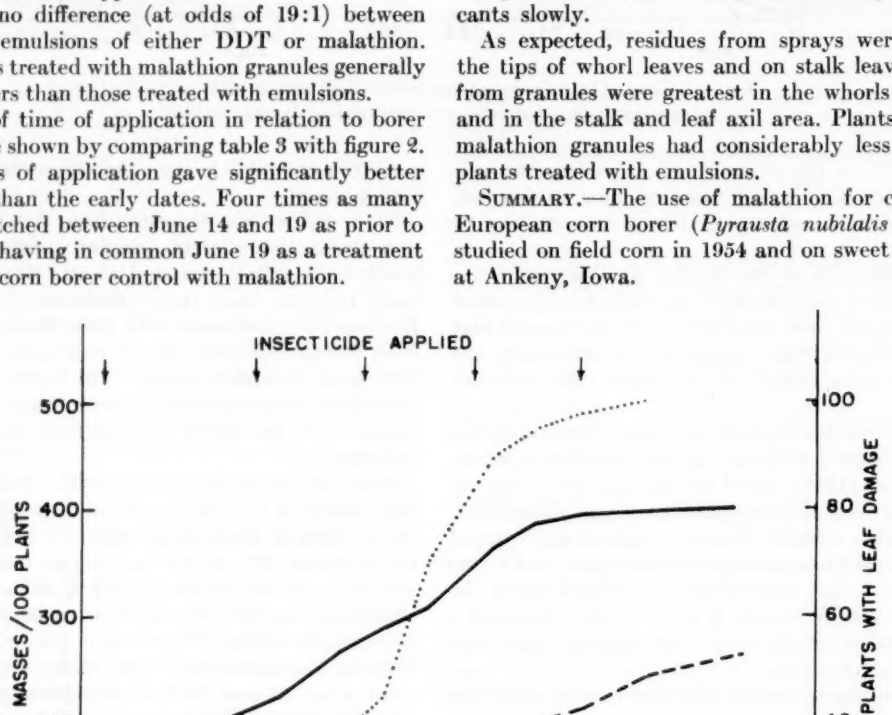

Fig. 2.—Time of insecticide applications to sweet corn in relation to corn borer egg laying, hatching, and leaf-feeding damage in 1955.

Table 4.—Malathion residues in parts per million, on different parts of sweet-corn plants after treating for control of first-brood European corn borer larvae, 1955.

Application Dates (June)	Date Sampled (June)	Tips of whorl Leaves		Whorl Leaves		Stalk Leaves		Stalk and Leaf Axils	
		Granules	Emulsion	Granules	Emulsion	Granules	Emulsion	Granules	Emulsion
2, 9, and 14	14	0	168.3	24.3	16.3	0	58.4	8.6	0.6
2 and 9	14	0	1.3	0.5	0	0	0.1	0.4	0
9, 14, and 19	19	0.3	96.4	45.2	1.8	0.3	20.7	12.3	0.3
9 and 14	19	—	0.5	7.2	0.2	—	0.4	1.0	0
14, 19, and 24	24	6.5	263.2	120.9	0	0.7	35.7	5.3	0.8
14 and 19	24	0	0	3.3	0	0	0	5.3	0

Malathion sprays prepared from wettable powders gave significantly better borer control than sprays prepared from emulsifiable concentrates. Granules were not significantly better than emulsions.

Plots treated with 2 pounds of malathion per acre had significantly fewer borers than plots treated with 1 pound.

Multiple applications gave better borer control than single applications. Control was related to the time of application.

Much larger residues were found on plants treated with 2 pounds of malathion per acre than on plants treated with 1 pound. Emulsion sprays deposited more residue than wettable-powder sprays, but the residues from emulsions were less persistent.

Granule-treated plants had considerably less residue than plants sprayed with an emulsion, but the residue persisted longer.

Practically all the residue from any formulation was lost in 5 days. Borer control was related to the number of newly-hatched larvae on the plant at the time of application. Consequently, the rate of loss or deterioration of malathion on treated plants was apparently too rapid to leave sufficient toxic residue for good corn borer control.

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Screening Greenhouses with Insecticide-Impregnated Cloth for Thrips[1] Control[2]

EDWARD J. KARLIN and JOHN A. NAEGELE, *Department of Entomology, Cornell University, Ithaca, N. Y.*, and GEORGE V. JOHNSON,[3] *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

Control of minute insects in greenhouses by screening greenhouse vents with cloth of a mesh small enough to prevent passage of these pests has been considered impractical since such screening also materially interferes with the ventilation of the houses. Therefore the screening of greenhouses with cloth of a mesh large enough to allow for the passage of air and small insects was investigated, the cloth, however, having previously been impregnated with various insecticides possessing residual properties. It was felt that thrips, being weak fliers and relatively dependent upon air currents, would be carried against the screening on passing through the vents and thus pick up sufficient insecticide by contact to kill them after they entered the screened greenhouses.

During the summers of 1954 and 1955, cloth screening was erected at six greenhouse ranges in Nassau and Suffolk Counties, N. Y. Screening was positioned in late May and evaluated until approximately the middle of August. All screened greenhouses were devoted exclusively to the production of roses. Both cheesecloth with a mesh of approximately 25 threads to the inch and nylon cloth with a mesh of 28 threads to the inch were tested.

[1] Identified by J. D. Hood. The principal species collected in these studies was *Frankliniella tritici* (Fitch); also found present but not numerous were *F. fusca* (Hinds), *Thrips tabaci* Lind., *Taenothrips inconsequens* (Uzel), and *Microcephalothrips abdominalis* Crawf.

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[3] Grateful acknowledgment is made to Mr. Jerry Haas who assisted in obtaining the experimental data.

However, the use of nylon was soon discontinued chiefly because of the high initial cost. Malathion, dieldrin, and heptachlor were used for impregnation. Untreated cloth was also tested as a measure of mechanical control alone.

PROCEDURE.—Folded bolts of cloth 100 yards long and 1 yard wide were placed in a flat pan partially filled with an emulsifiable concentrate of the insecticide to be used. More insecticide was poured over the bolts which were repeatedly turned and refolded to insure complete saturation of the cloth. Malathion and heptachlor were applied as 25% solutions and dieldrin as a 15% solution. The freshly impregnated bolts were allowed to drain and dry in the sun, after which the cloth was stapled to the wooden frames of the greenhouses. The procedures for attaching the cloth to both top and side vents were essentially identical. One edge of the cloth was folded around short pieces of wooden lath at 1- to 2-foot spacing to reduce strain and tearing, and with the vent partially open, the lath and cloth were stapled at 9- to 12-inch intervals to the outside of the vent near its lower margin. The other edge of the cloth was folded repeatedly around additional lath strips and stapled to the header bordering the vent so that only a slight excess of cloth remained when the vent was fully opened. Stapling through several folds of cheesecloth without the use of lath strips was also tested but proved less satisfactory. Modifications of screening procedures were made as the situations demanded. On several houses, the side headers were capped with metal. In such cases, the cloth was stapled just below the headers directly to the vertical wooden sash bars which were spaced at approximately 1-foot intervals. In all cases, screening was so placed that an extra loop or pocket of cloth was left at the ends of vents and between vent sections to allow for opening and closing the vents.

Rose samples were taken at 4- to 5-day intervals. During 1954 and the early part of 1955 approximately 10 blossoms were cut from each house, immediately placed in cellophane bags, and taken to the laboratory. Since we were attempting to detect the greatest amount of infestation, roses which were apparently insect-injured were always selected if present. White and yellow varieties appeared more susceptible to thrips attack and were sampled whenever possible. Most of the roses were cut from side benches since those areas displayed the greatest amount of damage. Early in the study, all rose blossoms were removed from the cellophane bags and a count was made of any thrips that remained in the bags. Following the method suggested by Taylor & Smith (1955), the blossoms were immediately placed in wire baskets suspended over solutions of detergent and water inside of glass cylinders. Open vials containing turpentine were hung above the blossoms and the cylinders covered with glass plates. Theoretically, the turpentine fumes drove the live thrips from the blossoms into the detergent solution where they became "wetted" and sank. After several hours, the blossoms were removed and picked apart, petal by petal, and any dead or living thrips counted. Finally, the water in the cylinders was drained and filtered. All thrips found on the filter paper were counted and preserved for identification. This method was time consuming, however, and offered little information concerning the dead thrips which may have been killed by toxicant-treated screening, turpentine fumes or other causes. Early in 1955, a second procedure was introduced in an effort to remedy this defect. The sampling technique was modified so that the rose blossoms were picked apart within tall glass cylinders and dropped directly into detergent solutions below. The mixture was stirred repeatedly and "wetted" thrips sank to the bottom while the plant parts floated. The cylinder walls were constantly observed and any crawling thrips were washed to the bottom. Since the thrips were not inclined to fly, few if any, escaped from the cylinders. Finally, the plant parts were removed and the water filtered. All thrips recovered were counted and preserved. If desired, microscopical examination before preserving allowed the recently drowned thrips to be separated from those which were dead for some time, the latter appearing much shriveled and off-color. Any possibility of the count being killed by the turpentine fumes was removed. However, this procedure did not allow for an accurate separation of thrips which had been drowned from those recently killed by toxicant from the screening. Since there was no way to measure the amount of damage a thrips caused to a blossom before dying, all living or dead thrips found in roses were considered as harmful infestants in the analysis of data compiled throughout both years of this study.

As the 1955 season progressed, two more modifications in sampling were made. It was felt that thrips might escape from the cellophane bags while being transported. Therefore, 1-gallon waxed cardboard containers were partly filled with detergent and water in the greenhouse and the freshly cut roses immediately submerged. In the laboratory the roses were swished through the detergent solution which was then filtered. Comparative tests indicated that thrips could be recovered from those soaked whole blossoms with as great accuracy as that attained in any method employing the more time-consuming techniques of tearing the blossoms apart. Taylor & Smith (1955) also found that this method of sampling gave results comparable to those obtained by the more laborious "turpentine method" previously described. Thereafter, this procedure was exclusively employed. No differences in the results were apparent from the use of these several sampling procedures. Therefore, all numerical data obtained from these procedures have been treated together.

SIDE VENT SCREENING (1954).—The side vents of five greenhouses at two locations were screened during the summer of 1954. Dieldrin, heptachlor, and untreated cheesecloth were used. An unscreened house was maintained as a check at each location. Unfortunately, the overall level of infestation at the greenhouse range where untreated cloth was used was so light that no accurate measure of control could be obtained. Data from this range have not been included in table 1 which summarizes the results obtained with dieldrin- and heptachlor-treated screening and an unscreened house.

Both types of treated cloth gave results significantly different from the check and from each other, the dieldrin giving approximately 1.4 times better control than the heptachlor.

SIDE VENT SCREENING (1955).—Cheesecloth which had been treated with heptachlor or with malathion and

Table 1.—Effectiveness of screening side vents of greenhouses with cheesecloth impregnated with dieldrin or heptachlor. 1954.

SCREENING	AVERAGE NUMBER THRIPS/ROSE	APPARENT PER CENT OF CHECK CONTROLLED[a]
Dieldrin-treated	9.2	86[b]
Heptachlor-treated	28.0	60
Check-unscreened	67.0	

[a] Significant; calculated at the 5% level by means of the Multiple Range Test of Duncan (1953).
[b] This percentage was derived from the data for screened and unscreened greenhouses at the time sampling ceased which was at an earlier date than in the other greenhouses.

untreated cloth were compared with each other and with their respective unscreened checks during the summer of 1955. Table 2 indicates the results of this screening of side vents.

While some control apparently resulted, the results in the three screened greenhouses were not significantly different from their unscreened checks at the 5% level. This was even true of heptachlor which gave significant results in 1954.

The lack of significance despite the apparently high percentage of control achieved can only be ascribed to extreme variability in the numbers of thrips on different sampling dates. The different sampling dates were considered as replicates in computing all data for this paper. It may be assumed that a small number of thrips safely penetrated the cloth barrier on any given date. Therefore, when populations were small, the number of thrips which penetrated the screening might equal or surpass the number of thrips entering the check house and thus strongly influence the final computations. On days of high populations, only a small number of thrips safely penetrated the barriers compared with the numbers entering the check greenhouse.

TOP AND SIDE SCREENING (1955).—During 1955, five greenhouses had both the top and side vents screened. Three houses were screened, one each with untreated, malathion-treated, and heptachlor-treated cloth while dieldrin-dipped cheesecloth was used on two greenhouses. Four unscreened greenhouses, one at each location, were also sampled as checks. However, one of the dieldrin-screened houses and its respective check have been eliminated from the evaluations which follow because of complete mechanical failure of the screening. The metal caps on the headers of the side vents of this house were set directly upon concrete offering no surface to which cheesecloth might be stapled. An improvised system was devised to circumvent this obstacle but it failed to hold the cloth securely. Table 3 indicates the degree of control obtained from the screening of top and side vents of four greenhouses when compared with their respective unscreened checks.

Table 2.—Effectiveness of heptachlor- and malathion-treated and untreated cheesecloth as side vent screening on greenhouses. 1955.

SCREENING	AVERAGE NUMBER THRIPS/ROSE	APPARENT PER CENT OF CHECK CONTROLLED[a]
Untreated cloth	0.1	95
Unscreened check	3.1	
Heptachlor-treated	0.7	79
Unscreened check	3.6	
Malathion-treated	2.0	65
Unscreened check	6.0	

[a] Significance was calculated by means of the Multiple Range Test of Duncan (1953) and none of the results were significant at the 5% level.

Table 3.—Relative effectiveness of top and side vent screening of greenhouses employing heptachlor-, malathion-, and dieldrin-treated, and untreated cheesecloth. 1955.

SCREENING	AVERAGE NUMBER THRIPS/ROSE	APPARENT PER CENT OF CHECK CONTROLLED
Untreated cloth	1.4	73
Unscreened check	5.1	
Heptachlor-treated	0.6	88
Unscreened check	5.1	
Malathion-treated	1.6	84[a]
Unscreened check	10.3	
Dieldrin-treated	0	100[b]
Unscreened check	3.6	

[a] Significant at the 5% level; calculated by means of the Multiple Range Test of Duncan (1953).
[b] Significant; computed by an "F" test at the 5% level.

Malathion-treated cloth proved to give significant control as measured by a multiple range test in comparisons of greenhouses screened with malathion-treated, heptachlor-treated, and untreated cloth and their unscreened checks. Data from a greenhouse that had its top and side screened with dieldrin-treated cloth, and its unscreened check were analyzed separately because the sampling dates (replicates) differed from those in other greenhouses, and the dieldrin-treated cloth gave significant control.

DISCUSSION.—Certain facts become apparent when all the data recorded in 1954 and 1955 are considered together.

(1) Untreated screening never gave significant control and has been eliminated from further consideration. (2) Malathion-dipped cloth gave significant control when applied to both top and side vents but failed to give control as side-screening alone. This fact, plus problems of odor and handling noted by the rose growers and workers applying the screening, eliminated malathion from further consideration. (3) Heptachlor-dipped cloth when applied only to side vents gave significant control in one greenhouse but not in another. It failed to give significant control as top and side screening combined at a third greenhouse. Therefore, heptachlor-impregnated cloth cannot be recommended. (4) Of the four materials tested, only dieldrin-treated cheesecloth consistently gave significant control. This was true regardless of whether top and side vents or only side vents were screened. Information published by Ginsburg & Jobbins (1954) tends to substantiate the superiority of dieldrin for our purpose for it indicates a breakdown rate for dieldrin out-of-doors some 20 times slower than that for heptachlor. (5) While not checked experimentally, it is probable that respraying the cheesecloth with dieldrin once or twice during the

season would maintain or improve the control of thrips. (6) Greenhouse temperatures were increased approximately 10° F. as a result of screening, but such changes did not cause any noticeable reduction of flower quality.

Several subsidiary problems were investigated concurrent with the evaluation of the various screening tests. Tanglefoot-coated plates erected at the level of the top and at the level of the side vents trapped approximately equal numbers of thrips, thus emphasizing the need for screening at both levels. However, plates facing in one direction consistently caught greater numbers of thrips than those facing in the opposite direction. Either one or a combination of two factors appeared to be responsible. The plates with higher counts faced the prevailing winds and also faced weedy fields while a cinder-covered parking lot bordered the range in the opposite direction. The influence upon thrips infestation resulting from fields of flowers around a greenhouse was pointedly illustrated at another range surrounded by extensive areas of grass and wild flowers. Plowing this area caused a large overnight increase in the numbers of thrips in the greenhouses. However, the numbers of thrips in subsequent samples eventually dropped to an apparently lower level than that noted before plowing. Large daily fluctuations in thrips counts tend to indicate that in addition to the immediate location of the range, the strength and direction of the prevailing winds also has a marked influence on the dispersal of thrips which may be wind-borne for great distances.

An interesting speculation arises from the above considerations. Thrips were never seen on the foliage in rose houses and apparently were attracted directly to the blossoms upon entering a house. Our sampling procedure indicated that thrips were only found in partially opened blossoms, those normally cut the same day. This appears to negate the "bullheading" of closed blossoms by thrips as some growers claim. In any case, it would appear that spraying or aerosoling with residual-type insecticides for thrips control, as commonly practiced, may be highly ineffective. Most, if not all, of the thrips present are in the blossoms which normally are cut and removed from the greenhouse the day of treatment. Any residual toxicant on the foliage apparently would have little effect on thrips arriving subsequent to the time of spraying. Thus, screening as proposed in this paper may prove to be the only economical way to control thrips on greenhouse-grown roses.

SUMMARY AND CONCLUSIONS.—As a method of thrips control greenhouses in commercial rose production were screened on the side or top and side vents with cheesecloth impregnated with emulsifible dieldrin, heptachlor, or malathion. Thrips were expected to penetrate such screening but die from contact residual action once inside the greenhouses. Experimentation indicated that screening should be reinforced with lath and stapled to the wooden frames of the greenhouses for best results, the actual procedure used being modified to fit each situation.

All living or dead thrips found in rose blossoms were counted as harmful infestants in analyzing the data. This was necessitated by an inability to measure the extent of damage which an individual thrips caused before dying.

Only dieldrin-treated cheesecloth gave consistently significant control with side screening alone and with combined top and side screening. It is therefore recommended for thrips control.

Spraying the cloth during the course of the thrips season may be advisable to maintain or improve control.

No impairment of rose production was noted after an approximate 10° F. rise in temperature resulting from screening.

The location of the greenhouse range and the direction of prevailing winds both appeared to influence daily fluctuations in numbers of thrips.

It seems probable that spraying within greenhouses to control thrips may be ineffective, particularly where roses are grown since the blossoms are cut and removed daily thus negating residual effects on that part of the rose plant where thrips are known to occur most often if not exclusively.

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The Omnivorous Leaf Roller, *Platynota stultana* Wlshm., on Cotton in Southern California: Damage and Control[1,2]

E. L. Atkins, Jr.,[3] M. H. Frost, Jr.,[4] A. S. Deal,[5] and H. T. Reynolds[3,6]

Larvae of the omnivorous leaf roller (Atkins *et al.* 1956), *Platynota stultana* Wlshm., infested all the cotton inspected in Imperial County, California, during 1954 (Atkins 1954). It was estimated that approximately half of the 67,000 acres grown in this area was economically damaged, some to the extent of nearly one-fourth of the crop. The damage is caused by the composite effect of larval feeding on leaves, terminals, squares, and bolls. Leaf-roller populations were also found near Blythe, in the Palo Verde Valley, and in the Coachella Valley, and in many of the cotton-growing areas of Arizona, especially in the vicinity of Yuma.

The year-round growing season enjoyed in these areas enables the omnivorous leaf roller to maintain itself at extraordinarily high population levels on a succession of host crops: alfalfa, celery, citrus, cotton, lettuce, and sugar beets. Population levels in infested cotton fields ranged up to 700 worms per-hour-search basis (see "Methods"), as compared to a high of 150 worms, per-hour-search basis, on citrus in California.

This is not a new pest in southern California, as it has been known to injure citrus for many years (Woglum 1920). Alfalfa is a favored host plant, and the leaf roller (Smith 1955) was reported as causing economic damage to this crop in the Imperial Valley during 1948. Cotton was not grown in the area at that time. During 1952 and 1953 the worms were observed on cotton but not in sufficient numbers to warrant investigation.

In 1954, alfalfa hay fields sustained moderately high populations of leaf rollers, but there was little reduction in yields. The damage was largely confined to the terminals of the plants. Late alfalfa seed fields, however, did suffer serious damage, the flowers being so heavily webbed and rolled by the infesting larvae that the flowers were unpollinated. Crops on many of the six to eight thousand acres infested were either cut for hay or abandoned. There are some indications that these fields occasionally serve as breeding areas for the leaf rollers, from which they spread into cotton in late July and August. During August and September over 230 plots were treated and studied in the Imperial Valley. Damage and control are reported in this paper. The nomenclature, life history, and bionomics of this insect are reported elsewhere (Atkins *et al.* 1957).

Damage.—Damage to the cotton plant may be grouped into four classifications, namely, that of (1) the leaves, (2) terminal buds, (3) squares, and (4) bolls.

Leaf damage is generally of minor importance, especially on plants over 1 foot tall. The worms are comparatively small and the reduction in leaf area from feeding is usually of little consequence. Figure 1A shows a large cotton leaf that has sustained above-average damage. On young cotton, damage to the small terminal leaves inhibits proper development and growth of the plants. Webbing of the larger leaves on top of each other also retards plant growth.

Damage to the terminal bud of the cotton plant occurs in many late-planted fields having high leaf roller populations. The injury occurs principally on plants 12 to 18 inches tall. Secondary buds usually develop, causing a heavily branched plant, rarely with a central stem (fig. 1B, C). Although counts indicate about the same number of bolls on injured plants as on normal plants, fruit setting is delayed and the top set has less time to mature during favorable weather.

Damage to the squares undoubtedly causes some loss in yield, as many squares drop prematurely, particularly if the flower bud has been injured. The most common damage, however, consists of larval feeding on the bracts and sepals, which often leaves the flower buds and subsequent bolls partly or completely exposed. Some of these bolls become sunburned and either drop or do not mature normally.

Boll damage was observed in several different forms. Some feeding injury caused the loss of either the entire boll or at least one or two locks, while other feeding injury allowed the boll to develop and produce lint normally. The old flower petals are favored nests for leaf rollers (fig. 1D). The worms web the corolla to the bracts and the young boll. Often these young bolls are destroyed by small larvae entering at the apex of the boll. The most common damage is the surface scarring of the larger bolls (fig. 1E). This causes some shrinking of the damaged areas, failure of the affected lock to open properly, and subsequent loss of lint. The rest of the boll often develops normally. If the feeding is deep and the larva enters the boll, boll rot organisms enter the injury and destroy the damaged locks. Entrance holes (fig. 1F) and deep feeding pits at the base of a boll (fig. 1G) usually cause the loss of the entire boll.

It is not possible to name any one type of injury as causing the greatest damage to cotton. It is rather the composite effect of all the types of feeding that adversely affects yield.

Control.—The difficulties confronting a successful chemical program for controlling this insect on cotton are due to several factors. This insect is very hard to contact effectively with an insecticide because of the larval habit of concealing itself in a nest of webbing between plant parts or inside the protection of a rolled leaf. This is commonly true with many of the other leaf rollers.

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[3] University of California Citrus Experiment Station, Riverside.

[4] Formerly with the University of California Citrus Experiment Station, Riverside; now with the Hercules Powder Company, Riverside, California.

[5] University of California Agricultural Extension Service, El Centro.

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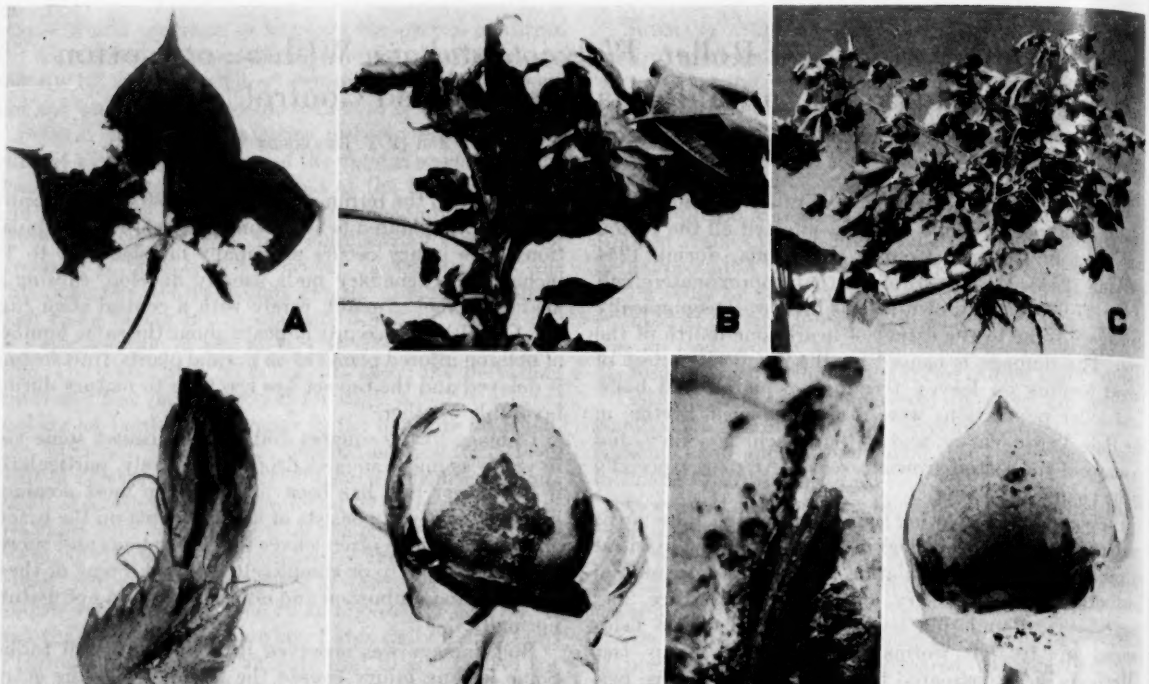

FIG. 1.—Damage to cotton plant by the omnivorous leaf roller, *Platynota stultana* Wlshm. A. Large cotton leaf showing above-average damage. B. Witches'-broom effect caused by larval feeding on cotton terminal. C. Heavily branched plant resulting from larval injury to central terminal and development of axillary bud growth. D. Injured flower petals webbed to corolla, bracts, and small boll. E. Larva on large boll, the surface of which is extensively scarred. F. Larva partially inside entrance hole on mature cotton boll. G. Deep feeding pits at base of mature cotton boll.

There are also wide differences between the maximum and minimum lengths of developmental time, some individuals requiring twice as many days to complete a life cycle as others (Atkins *et al.* 1957). In the Imperial Valley, where these tests were made, high temperatures or other unknown phenomena alter deposits of DDT and parathion insecticide quickly, making them ineffective. For instance, in the experiments reported here, surviving first-instar larvae were observed 24 hours after a parathion treatment and 48 hours after a DDT treatment. Insecticides are ineffective against the egg and pupal stages and in many instances are also ineffective against the adult stage.

METHODS.—Larval counts to determine the population level in a cotton field and the efficacy of an insecticide treatment were made on a per-hour-search basis. This method may be described essentially as follows. All the living larvae an observer can find anywhere on the plants during a given period of time are counted. As soon as the count is completed the number of living larvae found and the amount of time required for the count are recorded, and then both figures are converted to the per-hour basis. For example, if 20 living larvae are found in 6 minutes, the total larval population would be 200 worms on a per-hour-search basis. In the tests reported herein a uniform period of time was used for each replicate.

This method of determining a population of leaf rollers on a crop has frequently been termed the *time-search-basis* method. It was conceived by A. J. Basinger (1938) and has been successfully used for studies of lepidopterous larvae on citrus for over 20 years—for 8 years by the senior author. The effectiveness of an insecticide treatment is determined by comparing the reduction of living larvae on the plants of treated and untreated plots on a time-search basis. A pretreatment count is desirable but is not required with this method. It is a very efficient and rapid method of determining a population level and can be utilized to make field inspection counts of insects to determine when an economic population level has been reached.

Five other methods were explored for comparing the effectiveness of treatments: 1) counting the number of larvae found on a certain number of plants chosen at random per replicate, 2) counting the number of larvae found on a certain number of terminals picked at random per replicate, 3) counting the number of larvae found on a certain number of squares picked at random per replicate, 4) counting the number of damaged squares per random sample of squares per replicate, and 5) counting the total number of mature bolls and damaged bolls per linear foot of row just prior to opening of the bolls. The first three of these five methods can be used before, during, and after treatment. The samples of terminals and squares may be brought to the laboratory in paper bags for the subsequent counting of larvae. The fifth method may be utilized to determine relative production of the

Table 1.—Effects of various insecticide dusts applied August 13, 1954, for control of larvae of the omnivorous leaf roller on cotton near Brawley, California.

Material	Per Cent Dust Mixture	Pounds Toxicant per Acre	Number of Larvae After Treatment[a]	
			3 Days	1 Week
BHC	2	0.5	516	312
Cryolite	70	17.5	495	280
Isodrin	2	0.5	537	360
Malathion	4	1.0	441	304
Parathion	2	0.5	279	200
Perthane	10	2.5	378	208
Pyrethrins	0.2	0.05	501	252
TDE (DDD)	10	4.5	414	280
Toxaphene-DDT-sulfur	15–5–40	4.5–1.5–12	474	228
Check			489	280

[a] Per-Hour-Search Basis; for explanation, see "Methods."

treated and untreated plots. All six methods of observing cotton insecticide plots for leaf roller control are comparable, but the time-search-basis method is by far the most rapid.

EXPERIMENTAL TREATMENTS.—The results of the first test series of plots treated for finding an effective and practical insecticidal control for the larvae of the omnivorous leaf roller are shown in table 1. These insecticide dusts were applied August 13, 1954, with a high-clearance tractor-mounted Hardie Duster[7] equipped with a 30-foot boom covering 10 42-inch rows and making one round per treatment (2 acres) in unreplicated plots. Dusting was accomplished at 3 m.p.h. with a 1-m.p.h. wind and 85° F. temperature. The dusts were applied at the rate of 25 pounds per acre. The cotton was 30 to 34 inches tall, and the duster boom was operated 39 inches above the ground. Counts were made on a time-search basis, wherein all the living larvae found in 6 minutes of searching were recorded for each of four observations in each treatment area.

The results with parathion, Perthane, and toxaphene-DDT-sulfur dusts showed some promise. The reduction in larval population indicated in the plots after 1 week was due to the occurrence of pupation in the life cycle of the insect. Poor control in these tests was partly due to the fact that the leaf roller larvae were mostly fully grown at the time of treatment. Fully grown larvae are much more difficult to control than larvae in the younger stages. Very few insecticides are effective in destroying the pupal stage, and these usually only when used at relatively high dosages.

Table 2.—Effects of various insecticide sprays applied August 19, 1954, for control of larvae of the omnivorous leaf roller on cotton near Brawley, California.

Material	Pounds Toxicant per Acre	Number of Larvae After Treatment[a]	
		1 Week	2 Weeks
Aldrin	1.0	324	488
Strobane	4.0	302	348
DDT-toxaphene	2–4	155	310
Demeton	0.5	396	480
Dieldrin	0.5	329	477
Dilan	2.0	184	298
Endrin	0.53	261	413
Heptachlor	1.0	402	480
Parathion	1.0	73	73
Parathion-DDT	1–2	98	169
Parathion-Perthane	1–2	57	110
Parathion-TDE	1–2	63	114
Parathion-toxaphene	1–4	82	161
Rotenone-pyrethrum[b]	0.23–0.038	356	415
Check	—	388	483
L.S.D. at 5% level		71	95

[a] Per-Hour-Search Basis; for explanation, see "Methods."
[b] Botano: rotenone, 2.5%; other cubé resins, 5%; pyrethrum, 0.4%.

The results of the next series of tests, with sprays, are presented in table 2. In this test the materials were applied August 19, 1954, with a tractor-mounted Yellow-Devil Sprayer[8] having a boom covering eight rows and traveling at 3 m.p.h. Sprays were applied at the rate of 10 gallons per acre at a pressure of 60 p.s.i., using three No. X3 Spraying System hollow-cone nozzles[9] per row. Plots were eight 40-inch rows wide by 1320 feet long and were replicated four times for each treatment. The temperature ranged from 85° to 105° F. during the application, and the wind ranged from 1 to 4 m.p.h. The cotton was 24 inches tall and blooming. Leaf roller population was medium-heavy and occurred in all stages. Counts were made on a time-search basis, wherein all the living larvae found in 6 minutes of searching were recorded for each replicate of each treatment.

In this series of tests (table 2) parathion and the combinations of parathion-Perthane, parathion-TDE, parathion-toxaphene, and parathion-DDT were somewhat satisfactory treatments. DDT-toxaphene, Dilan, and Strobane were also significantly effective. Endrin, rotenone-pyrethrum, dieldrin, heptachlor, aldrin, and demeton were ineffective.

The results of another series of sprays are given in table 3. These insecticides were applied August 20, 1954, with airplane sprayer equipment covering eight 40-inch rows per $\frac{1}{4}$-mile pass, and four passes were made per treatment (3.2 acres) in unreplicated plots. Ten gallons of liquid were used per treatment (3.1 gallons per acre). The cotton was 30 inches tall. There was no wind, and the temperature was 90° F. at the time of application.

Parathion, methyl parathion, and the toxaphene-DDT combination were all effective in these tests (table 3). Strobane was ineffective. These applications should have been one-third less than indicated in the table. The high concentrations of toxaphene-DDT and Strobane caused

Table 3.—Effects of various insecticide sprays applied August 20, 1954, for control of larvae of the omnivorous leaf roller on cotton near Holtville, California.

Material	Pounds Toxicant per Acre	Number of Larvae After Treatment[a]		
		4 Days	1 Week	3 Weeks
Strobane	6.64	106	120	180
Methyl parathion	1.56	32	55	75
Parathion	1.56	48	60	60
Toxaphene-DDT	9.36–4.68	26	65	95
Check	—	176	115	205

[a] Per-Hour-Search Basis; for explanation, see "Methods."

[7] Hardie Manufacturing Company, Hudson, Michigan.
[8] Engine Parts Manufacturing Company, Cleveland, Ohio.
[9] Spraying Systems Company, Bellwood, Illinois.

Table 4.—Effects of various insecticide dusts applied August 24, 1954, for control of larvae of the omnivorous leaf roller on cotton near Holtville, California.

Material	Per Cent Dust Mixture	Pounds Toxicant per Acre	Number of Larvae After Treatment[a]	
			3 Days	1 Week
Calcium arsenate	70	30.2	261	337
Chlorthion	4	1.8	227	195
DDT-sulfur	10–40	5.0	156	155
Diazinon-sulfur	2.5–91	1.85–67.5	222	165
Parathion	4	2.12	78	87
Ryania	40	20.0	285	268
Ryania-isome[b]	15–0.5	8.07–0.29	266	295
Toxaphene-DDT-sulfur	15–5–18	12.3–4.1–14.8	150	170
Check			249	350
L.S.D. at 5% level			112	103

[a] Per-Hour-Search Basis; for explanation, see "Methods."
[b] Also known as Ryanexcel.

Table 5.—Effects of repeated applications of insecticide sprays applied for control of larvae of the omnivorous leaf roller on cotton near Brawley, California.[a]

Material	Pounds Toxicant per Acre	Number of Larvae[b]				
		Sept. 9	Sept. 16	Sept. 23	Sept. 29	Oct. 15
North Subsections—Three Treatments: Sept. 2, 10, and 16, 1954						
BHC-DDT	0.6–3	75	35	27	10	17
DDT	3	36	25	10	10	7
Parathion	0.5	69	47	40	10	42
Toxaphene-DDT	4.5–3	42	37	27	5	13
Check	—	201	220	235	105	73
L.S.D. at 5% level		53	47	50	15	35
South Subsections—Two Treatments: Sept. 2 and 16, 1954						
BHC-DDT	0.6–3	75	77	37	15	32
DDT	3	36	85	47	20	25
Parathion	0.5	69	145	85	47	82
Toxaphene-DDT	4.5–3	42	62	60	42	17
Check	—	201	227	227	125	77
L.S.D. at 5% level		53	66	32	49	24

[a] For these experiments replicate plots were divided into north and south subsections.
[b] Per-Hour-Search Basis; for explanation, see "Methods."

heavy shot-hole burning of the foliage by the spray droplets.

Results of a fourth series of tests are given in table 4. These test materials were applied August 24, 1954, with a Powerpak Duster,[10] a light, all-aluminum, gasoline-engine-powered unit that is carried on the back of a man. Dusting was accomplished at a normal walk, covering two rows at a time. Dusts were applied at the rate of 50 pounds per acre, holding the dust nozzles 6 inches above the cotton, which was 30 inches tall and was blooming. Plots were four 40-inch rows wide by 50 feet long and were replicated four times for each treatment (1/16.4 acre per treatment). The temperature was 95° F. at the time of applications, and no wind was discernible.

In this series of tests (table 4) the plots treated with parathion dust were the only ones showing significantly better control than the untreated area 3 days after treatment. However, counts made 1 week after treatment indicated that results with parathion, DDT-sulfur, Diazinon-sulfur, toxaphene-DDT-sulfur and chlorthion were all significantly better than in the untreated checks.

Another series of tests was applied September 3, 1954, in the Imperial Valley with a hand-compression sprayer at a pressure of 60 p.s.i., using the equivalent of 15 to 19 gallons of spray per acre. The application was made to both sides of each row. These plots were three rows wide by 100 feet long and were replicated three times for each treatment. Results with Shell OS-2046, applied at the rate of 9 ounces per acre, and with Pyrazoxon, applied at 8 ounces per acre, were highly significantly better than results in check plots after 1 week. Results with AC-528[11] at 8 ounces per acre, Am. Cyanamid 3911 at 9 ounces per acre, and Diazinon at the rate of 7 ounces per acre were somewhat significantly better than those in untreated check plots 1 week after treatment. However, the results with none of the above treatments were significantly better than in the untreated plots 2 and 3 weeks after treatment. Shell OS-2046, Pyrazoxon and Am. Cyanamid 3911 are systemic phosphates. AC-528 is a non-systemic phosphate pesticide.

Results of repeated applications of insecticide sprays to control the omnivorous leaf roller on cotton are given in table 5. These materials were applied in September, 1954, with Yellow-Devil Sprayer equipment with a boom covering eight rows. The high-clearance, tractor-mounted sprayer was operated at 3 m.p.h., and the sprays were applied at the rate of 10 gallons per acre at a pressure of 60 p.s.i., using three Spraying System No. X3 hollow-cone nozzles per row. Plots were eight 40-inch rows wide by 342 feet long and were replicated four times for each treatment. Temperature ranged from 85° to 107° F., and wind was 1 to 5 m.p.h. at the time of applications. The spray boom was adjusted to clear the cotton by 6 inches. The leaf roller population was medium-heavy and occurred in all stages. Counts were made on a time-search basis, wherein all the living larvae found in 6 minutes were recorded for each replicate of each treatment.

The plot area was randomized to provide four replications for each of the four treatments and for the untreated check area. Each of these replicates was further divided into a north and a south subsection. In both north and south subsections (table 5), results with toxaphene-DDT, DDT, and BHC-DDT were significantly better than in the untreated checks when each count was made, whereas results with the parathion treatments in both subsections were significantly better than in the checks except at the time of the October 15 count. When results of an insecticide treatment of the north subsection are compared statistically with the results of the same treatment in the south subsection, those of the north subsection treated three times at weekly intervals are significantly better at the 5% level than those of the south subsection treated twice at a 2-week interval in every instance except in the case of the toxaphene-DDT combination, which was not significantly better at the 5% level at the time of the October 15 observation.

It should be emphasized that the commonly used insecticides BHC and toxaphene are completely ineffective for controlling the larvae of the omnivorous leaf roller. This information has also been previously demonstrated in extensive screening of insecticides on citrus. Parathion, when used at the rate of 1 pound per acre, was the most effective material tested in this series. However, the Agricultural Code of California requires that an appli-

[10] Manufactured by Clapper & Glaser, Vista, California.
[11] Hercules Powder Company; 2,3-*p*-dioxanethiol *S,S*-bis(*O,O*-diethyl phosphoro-dithioate).

cation permit be obtained from the County Agricultural Commissioner's Office for the use of parathion and that each field treated be posted with appropriate warning signs when a pound or more of parathion is applied per acre. These signs state that persons should not enter the posted fields for 2 weeks after the treatment date. Consequently, a schedule of three weekly treatments would mean that workers must not enter these fields for such purposes as irrigating or weed control for a period of almost 6 weeks. The inconveniences and restrictions of these requirements are at present impractical. Therefore, it is necessary to report that DDT is the only practical and effective insecticide to use at present.

Table 6 presents additional information obtained from the DDT and parathion plots in this same series of tests studying the effects of repeated applications of insecticides for controlling the omnivorous leaf roller. In this table the total number of bolls indicates the production gain provided by the various insecticide treatments over the area left untreated. The figures show the accumulated effect of omnivorous leaf roller damage to leaves, terminal growth, and squares, as well as to the bolls. These figures are considered somewhat conservative, since the population levels in the untreated replicates are substantially lowered by the effective reduction of the large treated areas surrounding them (see table 5).

It is interesting to note from the data in table 6 that the leaf roller caused more damage to the bolls than the bollworm, *Heliothis zea* (Boddie).

Although the area receiving three weekly treatments of DDT at the rate of 3 pounds per acre per treatment produced slightly fewer bolls than that receiving two treatments, at a biweekly interval, of DDT at the rate of 3 pounds per acre per treatment, this difference was not significant. The authors think that three treatments are more desirable than two, when all factors are considered. Therefore, production and gross and net returns are presented on the three-treatment schedule only.

The area treated with DDT at the rate of 3 pounds per acre, applied three times at weekly intervals, gave a production of 1.63 bales of cotton per acre as compared to 1.32 bales per acre for the untreated area. This is an increase of 23.8% or 0.31 of a bale per acre over the untreated, using the rule-of-thumb figure of 10 bolls of cotton per linear foot of row as equivalent to one bale of cotton per acre. This production returned the grower $313.99 for the treated area and $254.28 for the untreated area, or a gross increase of $59.71 per acre.[12] The DDT treatments cost the grower $5.20 per application or a total of $15.60 for the three applications. Therefore, the grower received a net return of $44.11 per acre more for the treated acreage than for his untreated acreage.

On adjacent plots sprayed 2 weeks earlier in this same field (see table 2) the population level was 388 on August 25 and 483 on September 1. In this plot series the population level was 201 on September 9, 220 on September 16, and 235 on September 23. Therefore, a population of 200 to 235 worms per-hour-search basis was present during the treatment period, and this population level caused a decrease in yield of approximately 0.31 of a bale of cotton per acre, compared with that of the DDT-treated area.

Table 6.—Effects of repeated applications of DDT and parathion sprays on larval populations of the omnivorous leaf roller and on larval injury to cotton plants in test plots near Brawley, California.

Material	Pounds Toxicant per Acre per Application	Number of Treatments	Number of Larvae[a]	Number of Squares Damaged by Leaf Roller[b]	Number of Bolls		
					Total[c]	Damaged by[d] Leaf Roller Larvae	Damaged by[d] Bollworm
DDT	3.0	2	25.0	—	516	6.3	3.8
DDT	3.0	3	7.5	2.5	496	6.5	1.5
Parathion	0.5	3	42.5	4.2	434	11.0	9.5
Check	—	—	75.0	9.8	424	28.0	8.8
L.S.D. at 5% level			41.7	2.9	57.7	7.6	3.8

[a] On Oct. 15, 1954, one month after final treatment. (Per-Hour-Search Basis; see "Methods.")

[b] Number of squares from each 25-square sample chosen at random in each replicate (average of four replicates per treatment) on Sept. 29, 1954, 2 weeks after last treatment.

[c] Total number of bolls in each 30 feet of row on Nov. 18, 1954 (average of four replicates per treatment).

[d] Damaged mature bolls present on Nov. 18, 1954, in each 30 feet of row (average of four replicates per treatment).

DISCUSSION.—It is indicated from a study of all the insecticide tests applied and of all the population counts made that a population level of 100 larvae per-hour-search basis will cause serious economic damage to cotton. In other words, at this population level a grower will receive economic damage, and he will probable be ahead if he treats—at least he will not lose money by treating. On the basis of figures presented in tables 5 and 6, one is able to assume that a grower would break even at a population level of 45 to 50 worms per-hour-search basis provided that costs remained constant and that damage intensity was directly proportional.

It is further indicated from these data that the results with two treatments of DDT at the rate of 3 pounds per acre, applied 14 days apart, are significantly better than no treatment. However, two treatments may not provide the reduction in the population level necessary to prevent subsequent economic damage when treatment is applied earlier in the growing season. Three applications at weekly intervals greatly reduce the population level over two applications at biweekly intervals.

Additional tests should be made to determine 1) whether a timing interval of 7, 10, or 14 days is the most effective; 2) whether two or three applications are economically the more practical; and 3) whether dusts and sprays are equally effective or sprays more effective than dusts. Investigations should be made to determine whether other insecticides are more effective and more desirable than DDT.

SUMMARY.—The omnivorous leaf roller, *Platynota stultana* Wlshm., caused serious economic losses to more than 30,000 acres of cotton in the Imperial Valley of southern California during 1954. Some damage also occurred near Blythe, California, and Yuma, Arizona. Cotton is damaged by larval feeding on leaves, terminals, squares, and bolls. The composite effect of this larval feeding injury seriously reduces yields. The biology of

[12] Gross return to the grower was computed on the basis of 1470 pounds of seed cotton per bale of lint production per acre. Of this poundage an average of 58% or 852.6 pounds of seed at the rate of 3.25 cents per pound for $27.71, 34% or 499.8 pounds of lint at the rate of 33 cents per pound for $164.93, and 8% trash having no value gave a total return of $192.64 per bale of cotton.

the insect is an important factor in control. Results of screening tests, with 27 insecticides and with eight combinations of these materials for controlling this important insect on cotton are presented. DDT, parathion, methyl parathion, and Perthane were effective; Dilan, TDE (DDD), and chlorthion were promising; but the remainder of the insecticides, including the commonly used BHC and toxaphene, were ineffective. The omnivorous leaf roller is apparently capable of causing more damage to cotton bolls than the bollworm. For ultimate practical control a spray or possibly a dust program utilizing 3 pounds of DDT per acre per application and three applications at weekly intervals for medium to high infestations is indicated. The above treatment schedule demonstrated yield increase of 23.8% or 0.31 of a bale of cotton per acre over that of the untreated area. This provided the grower with a net return of $44.11 per acre more for the treated acreage than for his untreated acreage.

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Control of the Chicken Body Louse on Hens by Self-Treatment with Malathion Dust[1]

J. L. RODRIGUEZ, JR., and L. A. RIEHL, *University of California Citrus Experiment Station, Riverside*

The chicken body louse, *Menacanthus stramineus* (Nitz.), is a common pest of adult chickens in southern California. It remains on the bird all the time and creates a constant disturbance as it crawls about and nibbles on the feathers and the scaly materials on the skin. Edgar & King (1950) express the opinion that the body louse causes adult chickens more irritation than any other species of ectoparasite commonly encountered in the United States. The ultimate effect of the irritation on the growth of chickens and on egg production does not seem to be clearly established (Edgar & King 1950, Warren *et al.* 1948). Undoubtedly, however, body lice make chickens restless and nervous and are a source of unpleasantness and irritation to poultrymen.

A number of promising chlorinated hydrocarbon type insecticides were tested for control of lice on chickens in experiments conducted by Alicata *et al.* (1947), Telford (1945, 1947), Edgar *et al.* (1949), Smith (1952), Moore & Schwardt (1954), and Fairchild & Dahm (1955). Very satisfactory control was obtained with several of the materials and these have been used extensively by poultry growers.

Draper *et al.* (1952) found that residues of DDT ingested with feed appeared in eggs and in fat tissues of the hen. Fairchild & Dahm (1955), investigating residues of dieldrin and lindane from application of dusts to part of the plumage by the "pinch method," found residues (in parts per million of tissue) of lindane in fat and liver and of dieldrin in fat. Residues of lindane decreased with time, but those of dieldrin persisted for the observation period of 12 weeks.

Creighton *et al.* (1943) directed attention to the possibility of incorporating an insecticide in the dust bath to obtain distribution on the chicken's body and plumage as it dusted itself. Edgar *et al.* (1949) reported favorable results from the application of insecticidal dust to the litter.

Malathion was one of several promising synthetic organic insecticides tested by Moore & Schwardt (1954) against external parasites of chickens. A single spray was applied to the roost, or to the roost and selected surfaces of the house. The results obtained with malathion indicated definite promise for the general control of chicken ectoparasites.

Vincent *et al.* (1954) demonstrated that the application of malathion to the litter was very effective for the control of the northern fowl mite, *Bdellonyssus sylviarum* (C. & F.), and presented evidence to show that chickens were not affected deleteriously by direct application of dusts containing as much as 25% malathion, or by the addition of 50 p.p.m. of malathion to the feed, and that the taste of the eggs was not altered.

The findings of Vincent *et al.* (1954) are supported by intensive toxicological studies by March *et al.* (1956) on the fate of malathion in the chicken hen. Malathion was administered to the chickens (1) in the feed (100 p.p.m.) for 15 consecutive days, (2) by complete external coverage with a single application of 0.5% spray (112 p.p.m. of body weight), and (3) by a single intraperitoneal injection (178 p.p.m. of body weight). No gross toxic effects were noted in either the fed or the sprayed hen, and even the injected birds were not prostrated. The maximum total residues found in any of the tissues or eggs

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The pesticide chemicals discussed may not be used unless a tolerance has been established or an exemption from the requirement of a tolerance has been granted for each specific use, irrespective of the information contained in this report.

examined were less than 3 p.p.m. for birds given the feed and only 0.3 p.p.m. or less for sprayed hens; residues decreased rapidly after exposure to malathion was terminated. Evidence indicates that 90% or more of the total residues found are water-soluble metabolites and degradation products of malathion and that it is probable that the major part of these are relatively nontoxic.

The available evidence suggested the desirability of testing malathion for the control of lice on chickens. Control of ectoparasites on chickens by pesticides applied to the litter of the pen had been demonstrated (Edgar *et al.* 1949) and certain advantages were apparent for this method: (1) considerable savings in labor and time; (2) a minimum of activity and handling likely to excite and disturb chickens; (3) elimination of risks from wetting the birds with spray; (4) a minimum of dust particles that could be inhaled; (5) good distribution of insecticide through self-treatment by energetic, high producing hens; and (6) good likelihood of the presence of insecticide past the incubation period of the louse egg.

MATERIALS AND METHODS.—The experiments reported were begun in May, 1955. Initially, malathion dust was applied to the entire surface of the litter on the floors of the pens. It was noted that the hens chose specific areas of the floor in which to wallow and dust themselves. Restriction of distribution of the malathion dust to the wallows would obviously simplify application, save time and insecticides, and reduce hazards associated with the presence of malathion. An adaptation of this method, consisting of the placing of dusting material in dust-bath boxes built for the purpose and suitably located offered certain advantages for experimental purposes and perhaps for practical ones also.

Application to Entire Floor Litter Surface.—Ten pens, each containing approximately 350 hens, were included in the experiment. Each pen had from 3 to 10 inches of litter on a concrete slab. In general the litter was loose, dry, and relatively free of wet or caked material.

Five treatments were compared: (1) 1 pound of 4% malathion dust per 20 square feet of floor, (2) 1 pound of 4% malathion dust per 40 square feet, (3) 1 pound of 2% malathion dust per 20 square feet, (4) 1 pound of 1% malathion dust per 75 square feet, and (5) checks, untreated. Each treatment was used in each of two pens assigned at random.

The required amount of malathion dust was spread on the litter by hand, reasonable attention being given to covering the floor space evenly and completely. Care was taken to minimize the amount of dust in the air. Hens were left in the pens during application of the dust, but troughs for feed and water were removed. Eggs, particularly those on the floor, were gathered prior to treatment. Nests and roosts were not treated.

Observations to estimate the louse infestation were made before treatment and each week thereafter to 120 days. For each count, a total number of hens equivalent to 10% of the birds in the pen were caught singly, at random, and examined carefully for lice around the vent, in the fluff, and under the wings. The presence or absence of lice was tallied, and the results were expressed as percentage of hens infested.

Wallows.—For tests with wallows 10 pens were used, each containing about 250 hens. Five treatments were compared, namely, malathion dust at 2, 4, and 10% concentrations, respectively, applied to wallows, only, at the rate of 10 pounds of dust per pen; wallows untreated but 2.5 pounds of 1% malathion dust spread in each of the four corners of the pen; and no treatment (checks). Each treatment was used in each of two pens assigned at random. The labor required about 5 minutes per pen. Procedures for application of malathion and estimates of the louse infestations were similar to those used for treatments involving the entire floor surface. Hens were examined before treatment and each week thereafter to 44 days, when the number of hens was reduced considerably by extensive culling for commercial reasons.

Dust-Bath Boxes.—For these experiments boxes were constructed, placed in the pen, and filled with selected dusting material to provide a dust bath as a source of insecticide for self-application by the chickens. The boxes were 3 feet long, 2 feet wide, and 1 foot deep. A layer of 3 to 4 inches of dry litter of fine texture, taken from the pen, was placed in each box.

Two experiments were conducted with the dust baths. Eight pens, each containing about 250 hens, were used in the first experiment, and there were four treatments, each replicated twice. The dust-bath boxes were placed in the pen at predetermined locations, and insecticide was put into the dusting material as the dust bath was set up. This did not give the chickens an opportunity to become accustomed to the dust bath and to start to use it before the insecticide was added. The chickens did not seem to adjust readily to the dust baths, perhaps because they were new and strange or because of the insecticide.

Six pens, each containing about 250 hens, were used in the second experiment, and there were three treatments, each replicated twice. The dust baths, containing untreated dusting material, were set up in the pens at sites (wallows) which the hens has selected for dusting themselves. The hens were allowed 3 weeks to become acquainted with and to accept the bath as a site for dusting. When the insecticide was added, the chickens began to use the dust baths within an hour. The dusting material was remixed by stirring each week.

The treatments used in the first experiment were the checks and three concentrations of malathion, 1, 4, and 10%, respectively, on dust; in the second experiment the 1% malathion dust treatment was not used. Irrespective of the concentration of malathion, 1 pound of the formulated dust was mixed with the litter material in the bath. In each of the treatment pens a dust-bath box was provided for each 100 or fewer chickens, no boxes were put in the pens that were used as untreated checks. Chickens, eggs, troughs for feed or water, and other equipment were left in the pens during the respective applications of malathion to the dust baths.

The procedures used to estimate the louse infestation were similar to those described previously. Hens were examined before treatment and after, to 44 days in the first experiment and to 35 days in the second experiment. Observations were terminated because the number of hens was reduced by extensive culling for commercial reasons.

DISCUSSION OF RESULTS.—Data on control of the chicken body louse by malathion are shown in table 1 for application to the litter of the entire floor, in table 2

Table 1.—Average percentages of White Leghorn hens infested with the chicken body louse at intervals after treatment of the entire floor litter surface of pens with single applications of malathion dust at various concentrations and dosages.

Days After Treatment	Per Cent Lice-Infested Hens After Treatment			
	4% Dust		2% Dust	1% Dust
	(1 Lb./20 Sq. Ft.)	(1 Lb./40 Sq. Ft.)	(1 Lb./20 Sq. Ft.)	(1 Lb./75 Sq. Ft.)
0[a]	100	100	100	100
1	0	0	0	95.45
7	0	0	0	38.44
14	0	0	0	20.64
21	0	0	0	15.71
28	0	0	0	43.34
33	0	0	0	60.00
41	0	0	0	85.00
49	0	0	0	89.50
57	0	0	0	100
63	0	0	0	100
70	0	0	0	100
77	5.0	11.0	15.00	100
84	23.0[b]	30.0[b]	50.00[b]	—
91	34.0[b]	75.0[b]	96.68[b]	—
96	71.0[b]	71.0[b]	100[b]	—
105	90.0[b]	100[b]	100[b]	—
112	97.0[b]	100[b]	100[b]	
120	75.0[b]	100[b]	100[b]	—

[a] Pretreatment count. Untreated (check) hens were examined on each date and were all 100% lice-infested.
[b] Value for one replicate (no infestation in other replicate).

for application to wallows created and used by the hens for dusting, and in table 3 for dust-bath boxes constructed and placed in the pens. The pretreatment counts show that body lice were present on all the hens examined. In each case infestations continued on fowls of the untreated checks throughout the course of the experiment.

No lice were found within 70 days after application for three of the treatments shown in table 1. Although the fourth treatment provided some control, the relative inefficiency may be due either to the lower concentration of malathion in the dust or to the lower dosage rate.

Table 2.—Average percentages of White Leghorn hens infested with the chicken body louse at intervals after spot treatment of floor litter of pens (wallows and corners) with single applications of malathion dust at various concentrations.[a]

Days After Treatment	Per Cent Lice-Infested Hens After Treatment			
	10% Dust in Wallows	4% Dust in Wallows	2% Dust in Wallows	1% Dust 2.5 Lbs. per Corner
0[b]	95	96	24	100
1	0	11	6	—
7	0	0	0	8.0
14	0	0	0	16.7
21	0	0	0	8.33
27	0	0	0	—
30	0	0	0	—
37	0	0	0	—
44	0	0	0	—

[a] Malathion dust applied at the rate of 10 pounds per pen.
[b] Pretreatment count. Untreated (check) hens were examined on each date and were all 100% lice-infested.

Table 3.—Average percentages of White Leghorn hens infested with the chicken body louse at intervals after application of malathion dust at various concentrations to dust-bath boxes placed at random or at predesignated sites (wallows), with or without interval for hen acceptance.[a]

Days After Treatment	Per Cent Lice-Infested Hens After Treatment		
	1% Dust	4% Dust	10% Dust
Without Interval for Hen Acceptance of Dust-Bath Boxes			
0[b]	100	100	100
7	90	23	38
14	65	35	12
21	85	44	3
27	64	53	0
30	93	86	0
37	85	85	0
44	89	87	0
After Interval for Hen Acceptance of Dust-Bath Boxes			
0[b]	—	70	100
7	—	1.7	0
14	—	0	0
21	—	0	0
28	—	0	0
35	—	0	0

[a] Malathion applied at 1 pound of formulated dust per dust-bath box per 100 hens.
[b] Pretreatment count. Untreated (check) hens were examined on each date and were all 100% lice-infested.

The data of table 2 show that control in dust wallow treatment was equivalent to that of entire floor treatment. This suggests that if may not be necessary to treat the entire floor surface in practical control efforts to control the chicken body louse. No lice were found in pens in which the wallows, only, were treated with dust containing as little as 2% malathion. Considerable reduction of lice was obtained with the treatment in which dust containing 1% malathion was applied to the corners of the pen. The observations were discontinued at 21 days because the chickens were removed for commercial reasons. The results suggest that satisfactory control might be obtained with a concentration of 1% malathion in 2 applications at an interval of 14 days.

Comparison of the results obtained with dust containin 4% malathion for table 3 shows the value of placing the dust baths in the pens at sites selected by the hens for dusting, and of allowing an interval for the chickens to become acquainted with and to accept the boxes as dusting sites before application of malathion. In the first experiment with dust-bath boxes, results for the 10% dust illustrate the delayed acceptance of the boxes. When the hens began to use them a sufficient concentration of malathion remained to provide control from the 10 but not the 4% concentration. In the second experiment, self-dusting immediately after the application of malathion to the dust bath provided control with dust containing as little as 4% malathion.

Economies in time, labor, and insecticide are advantages observed for the dust-bath box treatments as compared to treating either the existing wallows or the entire surface of the floor. While the amount per pen is reduced when the insecticide is put in a dust bath, the concentration per unit of dusting material is several times greater than application to all or part of the litter. Loss of in-

secticide through the action of fresh droppings, water, addition of lime to litter, and activities of the chickens is minimized with material in boxes, and these can be placed at a safe distance from nests and troughs for feed or water. The amount of material containing insecticide is distinct and separate and is confined.

SUMMARY AND CONCLUSIONS.—Malathion was tested for control of the chicken body louse, *Menacanthus stramineus* (Nitz.), on White Leghorn hens. Dust formulations containing 1, 2, 4, or 10% malathion were applied manually to the surface of all or part (wallows) of the floor litter of chicken pens, or were put in dust-bath boxes. Distribution of malathion on the chickens was obtained by means of self-treatment accomplished by the natural dusting activities. The effects of the treatments on infestations of the chicken body louse on the hens were observed. Results demonstrate the high insecticidal efficiency of malathion against the body louse, and the practicality of the application method.

No lice were found after application of dust containing concentrations as low as 2% malathion (1) within 70 days, when 36 pounds were used per pen for the entire floor litter surface at 1 pound per 20 square feet; or (2) within more than 44 days, when 10 pounds per pen were put in the wallows only (either dosage equivalent to 4 pounds of material per 100 birds).

When dust-bath boxes were used, control of the chicken body louse for more than 35 days was obtained with concentrations as low as 4% malathion used at 2 to 3 pounds of dust per pen or 1 pound of material per 100 birds.

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The Effects of Temperature and Stage of Life Cycle upon the Toxicity and Metabolism of DDT in the House Fly[1]

J. J. MENN, E. BENJAMINI and W. M. HOSKINS

The finding that numerous strains of resistant house flies, *Musca domestica* L., and other insect species also, convert a large portion of absorbed DDT into DDE and other unidentified metabolites has caused some workers to conclude that this metabolism is a major factor in the resistance (Metcalf 1955, Hoskins & Gordon 1956). Others have felt that the metabolic changes occur more extensively in resistant individuals because these live longer after exposure. This conclusion seems to rest upon the following observations and deductions: a) resistant and susceptible flies metabolize about the same fraction of absorbed DDT, the actual amounts concerned being quite different (Lindquist *et al.* 1951) though a larger body of data indicate otherwise (Sternburg *et al.* 1950 Perry & Hoskins 1951); b) metabolism does not seem fast enough to protect an exposed individual (Winteringham 1952); c) surviving resistant flies may contain enough unaltered DDT to kill many susceptible flies if it were applied topically to them, but surviving susceptible flies contain only small amounts (Perry & Hoskins 1951, Tahori & Hoskins 1953); d) severely affected insects will recover in a few minutes if warmed to a higher temperature within the biological range and quickly revert to the intoxicated condition if cooled again at any time within a period which may be many hours with some insects (Vinson & Kearns 1952, Tahori & Hoskins 1953). Babers and Pratt (1953) concluded from data showing little dehydrochlorination in resistant flies treated with very large doses of DDT that the cause of resistance is quite unknown.

Some recent reviewers have stated the opposing theories without attempting a critical evaluation of either

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for the case of the house fly (Metcalf 1955, Wigglesworth 1955, Winteringham & Barnes 1955). With almost all other species the data are too scanty to permit an evaluation of possible mechanism(s) of resistance. In an effort to throw more light upon this important question the metabolism of DDT and the retention of unchanged DDT have been studied quantitatively in larvae and adults of two strains of *Musca domestica* which differ widely in their susceptibility to DDT. Additional pertinent data have been secured from analyses of flies held at three temperatures after exposure to the toxicant, which have revealed the differences in relative rates of metabolism in susceptible and resistant flies.

STRAINS OF FLIES, MATERIALS AND PROCEDURES USED.—The susceptible Stauffer strain was obtained from the Stauffer Agricultural Research Laboratory at Mountain View, Calif., in 1953 and has been reared in the insect toxicology laboratory at Berkeley since then. The stock culture has never been exposed to any insecticide. The adult females are used routinely in bioassay for insecticidal residues and have shown a uniform susceptibility, e.g., the LD_{50} values at 25° C. for DDT by the small vial method (Hoskins *et al.* 1952) have varied only from 2.5 to 3.0 μg. during the several months' duration of the present work. The resistant flies used were from the same Bellflower colony which has been maintained in the laboratory since 1948, with occasional selection of resistant individuals by exposure of adults to DDT. During the time of the present work the LD_{50} varied from 160 to 190 μg. per vial. For convenience these will be referred to hereafter as the S and B strains.

The larvae were reared on the Ralston Purina fly medium, 400 grams of which were mixed with 700 ml. water in a glass jar 22 cm. wide by 12 cm. high. In the larval exposure tests the required amounts of 50% DDT wettable powder to give 25 p.p.m. or 2000 p.p.m. DDT in terms of dry components were added to the water and thoroughly mixed through the medium. Fly eggs, after rejection of those that floated, were added at the rate of 0.3 ml. (approximately 2500) to a small pit in the center of the breeding jar and covered with a little medium. The temperature in the rearing room was kept at 25° C. and 60–70% relative humidity.

Adult flies were kept in the same rearing room in screen cages 1 foot on an edge. Those for testing were given sugar and water, but the breeding colony was fed milk in addition. Exposure to C.P. p,p'-DDT was made with 3- to 6-day-old females by the small vial method in which the DDT is dissolved in a thin layer of light spray oil on the inner surface of a shell vial. After exposure for 1 hour at 15°, 25° or 35°C., the flies were removed from the vials and kept at the same temperature and approximately 60% relative humidity in pint glass jars with sugar and water. Observation of knockdown and mortality was made at intervals as indicated later. From log concentration-probit plots, the LD_{50}'s and slopes of the log dosage-probit lines, hereafter called ld-p lines, were calculated (Hoskins & Gordon 1956).

At intervals larvae were selected at random from a pot (200 in case of 25 p.p.m. DDT in the diet and 25 in case of 2000 p.p.m.). They were washed in water and placed in 50% saturated NaCl solution for an hour to clear the gut, as shown by darkening of the salt solution and absence of color in the gut. Then they were shaken in benzene for 1 minute to remove any toxicant still adhering to the outside. After grinding with anhydrous sodium sulfate the macerated tissues were extracted by shaking for 1 hour with 40 ml. benzene. The combined extracts were shaken with 20 ml. concentrated sulfuric acid for 10 minutes and then with 20 ml. 10% fuming sulfuric acid for 30 minutes. After further washing with water, with sodium bicarbonate solution and finally again with water, the benzene was evaporated on a steam bath. Acetone was added and evaporated to ensure removal of all benzene. The procedure of Schechter *et al.* (1945), hereafter called the Schechter-Haller or S-H method, for DDT and DDE was then used with the modification that the final volumes were 1.5 ml. benzene plus 3.0 ml. of 10% sodium methylate. Readings were made with a Beckman DU Spectrophotometer at 596 and 540 millimicrons, from which the amounts of DDT and DDE were calculated as explained by Knudson *et al.* (1940) or by Perry & Hoskins (1951).

The procedure with adult flies was identical from the point when they were ground with anhydrous sodium sulfate. In all tests an equal number of larvae or of adult flies never exposed to DDT were carried through the same process and the amounts of apparent DDT or DDE found were subtracted in calculating the DDT and DDE present in the treated insects. Similar corrections were applied to external rinses of the flies, the interior of the exposure vials and the beakers in which exposed flies were confined.

RESULTS.—*a) Metabolism of DDT and mortality resulting from larval exposure.*—The objectives in the larval tests were on the one hand to expose larvae of the susceptible and resistant strains to the same amount of DDT, and on the other hand to expose them to amounts leading to equal mortality. Preliminary tests showed that 25 p.p.m. DDT in the diet caused no mortality at any stage with the resistant B strain and none in the larval stage of the susceptible S strain, but approximately 30% of these flies failed to emerge properly or died within a few hours after emergence with typical DDT "jitters." With 100 p.p.m. DDT in the diet approximately 80% of the S larvae were dead within 3 days. When 2000 p.p.m. DDT was included in the larval diet, no S larvae survived whereas the B larvae grew normally and pupated, but approximately 30% of the flies failed to emerge or died soon after emergence. The 25 p.p.m. and 2000 p.p.m. levels corresponded closely in effect upon the respective strains and hence were adopted for the larval exposures.

Analyses of larvae, pupae and adults were made at intervals through the life period. The data for the larvae at the end of feeding, i.e., fourth to fifth day, the pupae and surviving adults at few hours after emergence express the results adequately. These are given for the various exposures in table 1, in terms of μg. DDT or DDE per individual. The data are given as calculated to the second decimal place and may be taken conservatively as reliable to the first decimal place.

The data show that exposure of S and B larvae to 25 p.p.m. DDT led to more DDE and less DDT in the resistant strain at each stage. Since the differences are equal in magnitude, the simplest interpretation is that from equal total intakes the resistant strain formed more DDE

Table 1.—DDT and DDE in larvae, pupae and surviving adult *Musca domestica* resulting from larval exposure.

Strain	P.p.m. of DDT in Diet	DDT in μg. per Individual			DDE[a] in μg. per Individual			Per Cent Mortality of Adults
		Larva[b]	Pupa[c]	Adult[d]	Larva[b]	Pupa[c]	Adult[d]	
Stauffer	25	0.12	0.06	0.06	0.05	0.08	0.06	30
Bellflower	25	.02	.01	.01	.18	.12	.10	0
	2000	1.16	.05	.01	9.1	4.0	3.5	30

[a] Expressed in terms of DDT.
[b] Fourth or fifth day, approximately at end of feeding period.
[c] Fully darkened.
[d] Six to twelve hours after emergence.

and consequently contained less unaltered DDT. In the absence of information on total intake the possibility of other degradation reaction(s) of equal magnitude in each strain cannot be dismissed, of course.

Exposure of B larvae to 2000 p.p.m. DDT led to considerable DDT in the larvae (0.89 μg.) which decreased to a negligible amount in the surviving adults, although 30% of the pupae failed to emerge or the adults died shortly after emergence. These were not analyzed but they doubtless contained more unchanged DDT as found by Perry & Hoskins (1951) in comparison of dead and surviving flies after topical application. Exposure to 25 p.p.m. DDT, or to 2000 p.p.m. DDT, led in both cases to very small amounts of unchanged DDT in surviving B adults but only the latter exposure caused mortality. It is obvious from this situation that mortality is not correlated quantitatively with unchanged DDT in the adult stage.

b) Mortalities of susceptible and resistant female flies held at four temperatures after exposure to DDT by the small-vial method.—The object of these experiments was to measure the effect of the holding temperature upon the toxicity of DDT in light spray oil to the susceptible and resistant flies and to determine the relative heterogeneity in the reaction of these strains to DDT at the different temperatures as indicated by the slope of the dosage-mortality lines when plotted on log dosage-probit scales. Figure 1 shows the original dosage-mortality data for S and B flies at 15°, 25° and 35°. From these plots and from similar ones for other holding conditions after exposure the LD_{50}'s of table 2 were calculated. Since there were 25 flies per vial the LD_{50}'s per fly were obtained from those per vial by dividing by 25. This ignores the fact that only 80% of the total material in a vial is picked up by the flies (Hoskins *et al.* 1952), but this is not important for comparative purposes. The somewhat uncertain values for LD_{50} shown for B flies held 24 to 48 hours at 35° resulted from an unfortunate paucity of data obtained for this exposure and not from any indefiniteness in the flies' response. In figure 1, the heavy horizontal lines at 50% mortality show the 95% confidence limits of the respective LD_{50}'s calculated by the method of Litchfield & Wilcoxon (1949).

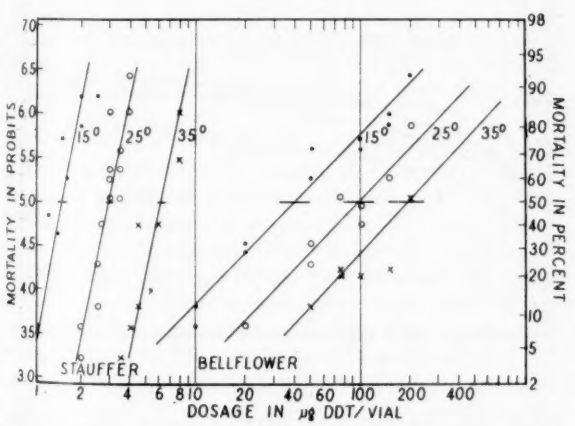

FIG. 1.—Dosage-mortality lines for female Stauffer and Bellflower flies exposed to DDT by the vial method.

Table 2.—Mortalities in terms of LD_{50}'s for susceptible and resistant flies exposed to DDT by the small vial method and held for indicated intervals at given temperatures.[a]

Strain	Hours Held at 10° C.	15° C.	25° C	35° C.	LD_{50} in μg. DDT per Vial	per Fly
Stauffer	24[b]				0.50	0.020
	24[c]				75	.030
		24			1.5	0.060
		48			1.3	.052
		24 →	→ 24		1.3	.052
		12 →	→ 24		1.5	.060
		6 →	→ 18		1.5	.060
			24		2.8	0.112
			48		2.7	.108
				24	6.3	0.252
				48	6.3	.252
				12 → ← 12	3.0	.120
				18 → ← 6	3.1	.124
Bellflower		24			200±	8.0±
		48			40	1.60
		24 →	→ 24		40	1.60
		12 →	→ 12		70	2.80
		6 →	→ 18		85	3.40
			24		96	3.84
			48		82	3.28
				24 or 48	195±	7.8±
				12 → ← 12	120	4.80
				18 → ← 6	94	3.76

[a] Arrows indicate sequence of temperatures.
[b] Held in vials at 25° for ½ hour, then at 10° for ½ hour.
[c] Held in vials at 10° of 1 hour.

The tests made with S flies held at 10° C. are interesting for two reasons; first, they show that the negative temperature coefficient of toxicity, as indicated by a lowered LD_{50}, still holds when flies are decidedly sluggish because of the cold and secondly, exposing in the vials for one half hour at 25° by increasing the rate of penetration (see later section for data on this point) resulted in a lower LD_{50} than when exposure and holding were entirely at 10°. These experiments could not be repeated with the B flies because they respond to the DDT so slowly at 10° that no mortality line could be determined.

A simple method for expressing the effect of temperature upon toxicity is the ratio of the LD_{50} at 35° to that at 25° and similarly for 25° and 15°. The temperature coefficients so measured, the so-called Q10 values are: Stauffer adult females 35°/25°, 2.1; 25°/15°, 2.2; Bellflower adult females 35°/25°, 2.4; 25°/15°, 2.0. The LD_{50}'s are those for 24-hour holding periods except for 15°, in which case those for 48 hours are used because toxic action was incomplete in 24 hours with the B flies and only an approximate value could be determined. It is evident that the temperature coefficient is almost the same for

the two strains and that all are in the range often found for biological processes.

It is of interest to note that the same result was obtained if S flies were held at 15° for the full period or only for 6 hours and then were transferred to 25°. With B flies held 6 hours at 15° before transfer to 25° the LD_{50} was within the range for 25° for the full period. But if the B flies were held at 15° for 12 hours before transfer to 25° the LD_{50} was intermediate between those for 15° and for 25° and moving them to 25° after holding at 15° for 24 hours did not alter the toxicity at all. This slower response of the B flies to a higher holding temperature probably is related to the observation that toxic symptoms developed in the S flies almost at once when they were put into the exposure vials at 15°, but in the B flies only after a few hours have elapsed. With both strains mortality increased up to nearly 48 hours.

A somewhat different result was obtained by holding first at 35° and then transferring to 25°. The S flies were affected slowly by exposure to DDT at 35° and mortality continued to increase for a long time, probably to about 24 hours. If transferred to 25° after 12 hours at 35° they had the same LD_{50} as if held continuously at 25°. At 35° the B flies were very quickly affected by exposure to DDT and the full mortality was reached with 6 hours. Transfer to 25° after 12 hours at 35° gave a final LD_{50} intermediate between those for 25° and 35°. If the period at 35° was reduced to 6 hours followed by holding at 25°, the LD_{50} was that for 24 hours at 25°. Thus a few hours at 15° appears to fix the toxicity for S flies but at least 12 hours at 15° are needed to influence toxicity for B flies. In contrast, holding S flies at 35° for 12 hours before moving to 25° had no effect on toxicity but it strongly influenced the final effect with B flies.

Differences were noticed between the susceptible and resistant strains with respect to the time of onset of symptoms of toxic action such as buzzing, rolling about and turning on the back. Stauffer flies became agitated almost at once after contacting DDT solutions at 15° and the symptoms lasted for many hours. Mortality however developed slowly and increased to about 48 hours. At 25° and especially at 35° the symptoms came on only after several hours except with the heaviest dosages and mortality continued to increase with time. The Bellflower flies were affected slowly at 15°. After 24 hours many flies were down but not dead. The negative temperature coefficient of toxicity was not evident at that time for the approximate LD_{50} determined as accurately as possible was very high. But by 48 hours it had dropped to the low value of 40 μg. per vial. On the other hand, exposure of the B flies at 35° caused very rapid development of symptoms and full mortality developed within 6 hours, unless the flies then were moved to a lower temperature.

In practical terms, a drop in temperature such as occurs during the night time had more effect in increasing kill of susceptible flies after exposure to DDT than with resistant ones and raising the temperature for a few hours gave more added protection to the resistant than to susceptible flies. Thus in fluctuating temperatures resistant house flies have an extra ability to survive exposure to DDT in addition to their usual tolerance as compared to ordinary flies at constant temperature. The results discussed above were all obtained from single exposures of limited degree and may not be applicable to continuous contact as with a residual deposit on the roosting area.

c) Effect of temperature upon the absorption of DDT and production of DDE following exposure of adult flies to a constant amount of DDT.—It was intended to expose each strain to an equal dose at 15°, 25° and 35° C and hold at the same temperatures. Preliminary tests showed that 3 μg DDT per vial gave sufficient internal DDT and DDE for satisfactory analyses and negligible mortality of the B strain females at each temperature. But the S flies could be used only at 35° because mortality was severe at the two lower temperatures. All tests were carried out with eight vials containing a total of 200 flies. Since the dosage used was arbitrary, the absolute magnitude of the amounts of compounds concerned is less important than the variation with temperature and time. For this reason the results are given in graphic form. Figure 2 shows the average amounts found in what were usually duplicate or triplicate runs.

The absorption of DDT from the oily solutions in the vials followed the same pattern as that observed in earlier work, i.e., there was rapid entry for the first 4 hours or so and much slower uptake thereafter (fig. 2A). Absorption increased with rising temperature but after about 24 hours there was no appreciable difference between the amounts taken in at 35° and at 25°. After 48 hours total absorption was about the same for all three

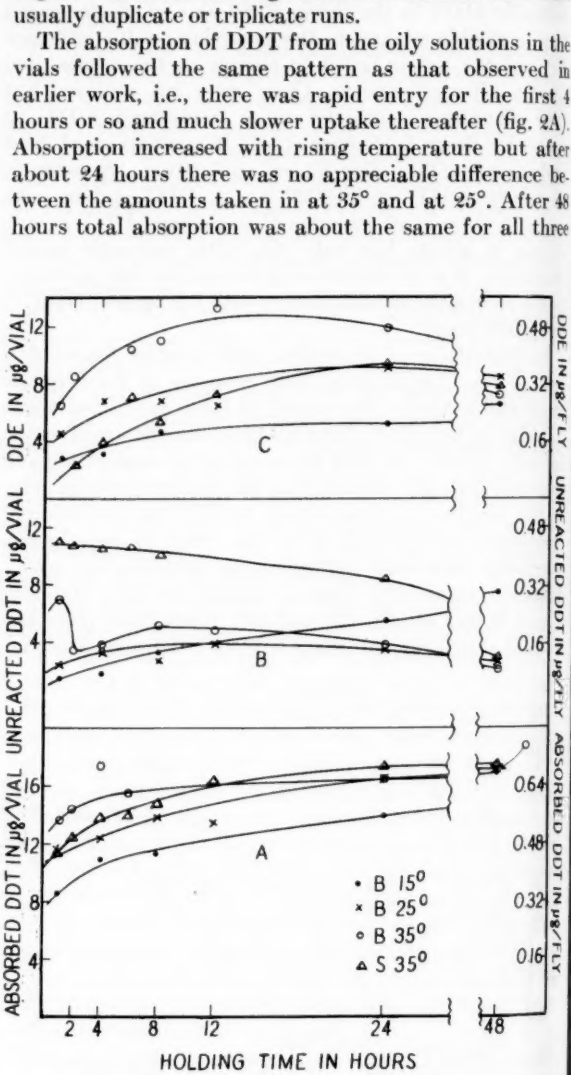

FIG. 2.—Changes in absorbed DDT, unreacted DDT, and DDE during holding period after exposure of S and B flies to DDT in oil by vial technique.

temperatures. Comparing the susceptible S flies and the resistant B flies it is clear that the latter absorbed appreciably more DDT for the first few hours but somewhat less thereafter. The differences were slight however.

A point of much interest is that the very marked decrease in uptake at low temperature found by Vinson & Kearns (1952) and Tahori & Hoskins (1953) after topical application of acetone solutions was much less evident with oil solutions of DDT. This indicates that whereas dry deposits of DDT are taken up very poorly at low temperatures, oily deposits such as result from use of oil sprays will be more effective because the DDT is absorbed only slightly less than at ordinary temperatures.

Turning to the data on DDE in the flies (fig. 2C), the amounts found increased markedly as the temperature was raised, though after 24 hours there was a slow decrease at 25° and especially at 35°. DDE concentration in S flies at 35° was below that in B flies at 15° for the first few hours but it continued to increase for 24 hours or so. The data on unaltered DDT in the flies are probably the most interesting of all (fig. 2B). The large amounts in the S flies and its slow decrease were in accord with the small amounts of DDE found in them for the first several hours. The B flies exposed and held at 35° contained a rather large amount of unaltered DDT at 1 hour in agreement with the rapid onset of symptoms of poisoning, but it fell precipitiously in the next hour and varied but little after that time. This effect was confirmed in check tests. No explanation can be offered except the suggestion that such a result would follow if the entering DDT encountered a region where it was rapidly metabolized 1 to 2 hours after the flies were removed from the exposure vials. Only cytochemical tests of a delicacy at present impossible would throw light on this point.

A similar but less marked change in amount of unaltered DDT may have occurred at 25° but unfortunately the analyses at 1 hour were highly discordant and a meaningful figure cannot be given. The plot shows the single analysis at 1 hour that appeared to be free from manipulative difficulties. After 24 hours about the same amount of unaltered DDT was present in flies held at 25° and at 35° and after 48 hours there was slightly more at the lower temperature. The slow formation of DDE at 15° and continued absorption of DDT caused the unchanged DDT in the body eventually to reach a high level, in fact at 48 hours it exceeded any found in the B flies at any other time or temperature. This behavior at 15° appears to be an exaggeration of the effect which was slightly apparent at 25°.

DISCUSSION.—The results of these experiments have added certain information bearing on each of the points mentioned in the first paragraph of this report. The idea that an equal fraction of absorbed DDT is metabolized by different strains is not borne out in either the larval or adult stage. Since all larvae grew at about the same rate and hence probably consumed equal amounts of medium, it is reasonable to assume that DDT intake was proportional to its concentration. The lower amounts of unchanged DDT in B larvae, pupae and adults resulting from exposure to 25 p.p.m. DDT in the diet are the result of greater metabolism in this strain. This conclusion is reinforced by the fact that the sum of unchanged DDT plus DDE is practically the same at each stage of the two strains, e.g., 0.17 vs. 0.20 in larvae, 0.14 vs. 0.13 in pupae and 0.12 vs. 0.11 in adults. This is an indication that DDE is the only metabolite formed in appreciable quantities.

Babers & Pratt (1953) have suggested that relative extents of DDT metabolism should be compared at equal degrees of toxic action. This may be done with S larvae exposed to 25 p.p.m. DDT and B larvae exposed to 2000 p.p.m. since neither amount caused appreciable larval mortality but each caused about 30% mortality at emergence. The ratio of DDE found in the body to DDT entering is a useful quantity for comparing the strains. Taking unchanged DDT plus DDE as the total entering, these ratios are for 4- to 5-day S larvae $0.05/(0.12+0.05)=0.29$; for B larvae $9.1/(1.16+9.1)=0.89$. Somewhat higher figures may be calculated in a similar manner for the pupae and adults. It should be noted, however, that in the case of the B strain there is evidence that much DDE left the larvae as shown by the following data on DDE content in successive days after hatching: 3 days, 6.0 μg; 4 days, 6.7 μg.; 5 days, 9.1 μg.; 6 days 5.0 μg.; 7 days, 4.5 μg.; 8 days (pupation), 4.5 μg.; 9 days, 3.5 μg. and no change thereafter until emergence on the 11th day. Whether the DDE decreased because it was metabolized further or because it was excreted is not known. In either case the true ratio of DDE formed to DDT entering would be somewhat greater than calculated above and the contrast between the susceptible and resistant strains is greater than indicated by the figures given. It will be noted that these fractions metabolized correspond to the "resistance index" suggested by Perry *et al.* (1953) as a measure of resistance.

In the case of exposed adult flies the data shown in figure 2 permit a direct calculation of the resistance index. In these tests exposure was the same and analysis was made on survivors only, though mortality was low in all cases. Using the suggested time of 2 hours the figures for 35° are $2.3/12.6=0.18$ for S adults and $8.7/14.5=0.60$ for B adults.

The role of speed of DDT metabolism is best illustrated by S and B adults exposed to the same amount of toxicant at 35° (cf. fig. 2). The high level of unchanged DDT maintained for many hours in the susceptible flies is in sharp contrast to the abrupt drop which occurred in the resistant ones. It may be calculated that the percentages of entering DDT metabolized during the critical first few hours were: at 1 hour, S 3.6%, B 48%; at 2 hours, S 15%, B 67%; at 4 hours, S 23%, B 74%. The figures for B flies at 25° and 15° were comparable. It is of interest to note that in a highly resistant strain of house flies Sternburg *et al.* (1950) found 96% of the entering DDT to be metabolized during the first hour and only slightly less during later periods.

The results just mentioned do not, of course, give any reason to dispute those cases in which metabolism in resistant organisms accounts for a smaller portion of the absorbed toxicant. In such instances storage in nonsensitive regions of the body is the most probable reason for low toxicity. The present data afford an illustration of this situation. Referring to table 1, it might be expected that the amounts of DDT present in the adults would govern mortality in that stage but the negligible amount resulting from 25 p.p.m. DDT in the larval diet caused no

deaths in case of the B strain but the equally small amounts left after exposure to 2000 p.p.m. killed 30%. The two situations differ sharply in the amount of DDE present in the adults. It may be suggested that the mortality when the larger amount of DDE is present is due to the same synergistic effect of DDE upon DDT reported by Tahori & Hoskins (1953). Regardless of the explanation, the data of table 1 illustrate the fact previously noted by them that the total amount of DDT in individuals of a given strain of insects is not a reliable indicator of the toxic effects to be expected. It is even less of an indicator of toxicity with different strains.

The reversible manner in which symptoms of poisoning by DDT may be brought on by cooling and removed by warming an exposed insect has led to doubt that detoxification of the chemical could be concerned in its effects. Two situations may be distinguished. Rapid alternation in temperatures with onset and disappearance of symptoms cannot be the result of any appreciable change in the total amount of toxicant in the body, for the known rates of metabolism, e.g., as shown in figure 2, are too slow. Such effects may be attributed to local changes in concentration or distribution which have not been measured as yet.

A change in sensitivity of insect larvae to DDT as the temperature changes was suggested by Yumasaki & Ishii (1954) on the basis of results from electrical stimulation of the crural nerve of the American cockroach in the presence of DDT. The significance in this connection of the water-soluble nerve stimulant found by Shankland & Kearns (1955) in the blood of DDT-treated cockroaches at certain stages of intoxication remains to be evaluated.

But the effects of changing the holding temperatures as done in the present tests can be correlated with variations in metabolic rates. Perhaps the simplest presentation of the data is in terms of changes in absorption and in metabolism as the exposure and holding temperature was altered. The following data taken from those used in constructing figure 1 refer to B flies exposed to 3 μg. DDT per 25 flies in the standard exposure vials.

Period of Holding (hours)	*Ratio μg. DDT Absorbed (35°/15°)*	*Ratio μg. DDT Metabolized (35°/15°)*
6	1.40	1.33
12	1.31	1.32
24	1.18	1.51
48	1.02	1.62

A sample calculation is that for 6 hours: DDT absorbed at 15°, 11.2 μg.; at 35°, 15.7 μg. (ratio 1.40): DDT metabolized (calculated as DDT absorbed less DDT found unaltered) at 15°, 8.2 μg.; at 35°, 10.9 μg. (ratio 1.33). Up to 12 hours the two processes were affected about equally, but thereafter the total absorption approached equality for the two temperatures and total metabolism became relatively much greater at 35°. If the calculation is made by intervals, i.e., for 12 to 24 hours and 24 to 48 hours, the changes are accentuated, for more DDT was absorbed at the lower temperature whereas more was metabolized at the higher temperature.

This means that if the insects survive the first several hours, they are in an increasingly favorable situation at higher temperatures since DDT is being metabolized faster than it is entering, and vice versa at low temperatures. It may be suggested that the same reasoning is applicable to the case of the American cockroaches treated topically with DDT solution by Vinson & Kearns (1952). They concluded that the relative changes in rate of absorption and rate of metabolism were in such a ratio that the high mortality at 15° could not be due to low metabolism at that temperature. It is obvious from their tables 2 and 3 that during the first 12 hours absorption exceeded metabolism at both temperatures. During succeeding intervals this situation underwent a reversal (with some inconsistencies in the data) and a recalculation for the interval 12 to 96 hours for both the 25 μg. and the 50 μg. dose gives the following increases during that period:

	Increase in Absorption		*Increase in Metabolism*	
25-μg. dose 15°	3.1	ratio 1:3.5	2.1	ratio 1:6.9
35°	10.9		14.5	
50-μg. dose 15°	8.9	ratio 1:2.3	7.5	ratio 1:3.8
35°	20.8		28.8	

Thus metabolism lagged behind absorption at the lower temperature but exceeded it at the higher temperature and if the cockroach lived through the first 12 hours, conditions for survival were increasingly more favorable at the higher temperature.

A recalculation of the data on absorption and metabolism by intervals shows the same situation for the house flies at three temperatures as cited by Tahori & Hoskins (1953). Of course, this argument is not an explanation of why normal acting flies at 35° can contain several times as much unchanged DDT as prostrate flies at 15°. But the fact that the steady metabolism of DDT continuing over a long time did not remove this internal amount gives reason to believe that it was stored in regions where it was neither toxic nor available for metabolic changes.

The foregoing discussion leads to the conclusion that survival will be favored by high temperature whenever conditions of exposure are such that after a short time the rate of detoxification, or perhaps excretion, exceeds intake of the toxicant. This situation is typical of single exposures, e.g., topical application or limited exposure to a treated surface, since the rate of penetration soon falls off at higher temperatures, but at low temperatures penetration continues more uniformly for a longer period. In other words, toxicity will have a negative temperature coefficient if the dose is moderate in size and given but once. But a heavy dose that kills before entry falls to a low rate will have a positive temperature coefficient. Cases in which all dosages are fatal and relative toxicities are measured in terms of time required for a certain kill come under the category of positive temperature coefficient, e.g., DDT or lead arsenate on Japanese beetle larvae (Fleming & Maines 1948), honey bees dusted with DDT powder (Guilhon 1946), mosquito larvae placed in concentrated DDT suspensions (Wiesmann & Zinkernagel 1946, Richards & Cutkomp 1946).

The present experiments were not concerned with continuous exposures, but the theory just presented indicates that whenever intake does not diminish after a time, the temperature coefficient of toxicity should be positive. The extensive work of Pradhan (1949) in *Tribolium castaneum* adults on DDT films illustrates this situation and his results with the web-spinning

Plutella maculipennis larvae show how an apparent reversal of the rule may arise because of changes in accessibility of the toxicant.

The slope of the log dosage-probit line (the ld-p line) is an indication of the degree of heterogeneity of the population in its response to a toxicant. The S and B strains of house flies are very different in this respect and they follow the usual rule, that the slope declines as resistance increases (Hoskins & Gordon 1956). The three ld-p lines for each strain as shown in figure 1 appear to be parallel and hence temperature does not affect the degree of heterogeneity in the reaction to DDT. The numerical values of the slopes may be calculated from the S function defined by Litchfield & Wilcoxon (1949) as $S=(LD_{84}/LD_{50}+LD_{50}/LD_{16})\div 2$ which gives the ratio of LD's for a change in mortality equal to one probit unit. Defining the slope as change in probit units per 10 fold change in dosage, a simple diagram will show that the following proportion holds: slope: log (10/1=1: log S. Hence slope = 1/log S. This expression is an improvement over the suggestion of McKenzie & Hoskins (1954) that slope be taken as 1/S, since it is the actual tangent of the angle between the dosage axis and the line when logs of dosage and probits are measured in units of the same length. Slopes may be determined graphically also by measuring the number of probit units that the ld-p line rises as the dosage increased 10 fold. Use of either method gives the following values of the slopes: S flies, 9.3; B flies 2.0.

The reliability of these values for the slopes may be found by calculating the confidence limits. The procedure of Litchfield & Wilcoxon (1949) gives the following limits for 95% confidence: S flies at 15°, slope lies between 7.9 and 11.1; B flies at 15°, slope lies between 1.6 and 2.6. Similar ranges hold for the other temperatures, except for B flies at 35°, in which case the slope is less certain because of few data. The foregoing discussion illustrates the value of recording LD_{50}'s, LD_{84}'s and LD_{16}'s or sufficient raw data so these may be calculated or a ld-p line drawn whenever toxicity data are recorded.

SUMMARY.—Inclusion of 25 p.p.m. DDT in the larval diet of susceptible (S) strain of house flies resulted in less than a microgram of DDT and of DDE in the adults and 30% of them died soon after emergence. The same amount in the larval diet of a resistant (B) strain resulted in much less DDT and more DDE in the adults and no mortality. Increasing the DDT to 2000 p.p.m. killed all S larvae. The B larvae from this diet contained over 1 microgram DDT which decreased to an inappreciable amount in the adults. DDE was 9 μg. in larvae decreasing to 3.5 in the adults. A synergistic effect of this DDE with the small amount of DDT probably accounts for the 30% mortality of the emerging adults.

LD_{50}'s for exposure to DDT in oil are for S flies: 15°, 0.052 μg.; 25°, 0.112 μg.; 35°, 0.252 μg. For B flies: 15°, 1.60 μg.; 25°, 3.84 μg.; 35°, 7.8 μg. The temperature coefficients calculated from these values are all negative and vary from 2.0 to 2.4. When exposed to DDT at 15° the S flies are quickly affected and the same mortality results whether they are held at this temperature for 24 hours or only for 6 hours and then changed to 25°. B flies are affected slowly at 15° and consequently holding at 15° for 6 hours plus 18 hours at 25° results in same mortality as when total exposure was at 25°. Twelve hours at each temperature gives a mortality of B flies intermediate between those for the two temperatures, but if held at 15° for 24 hours and then moved to 25° there is no further mortality. A few hours at 15° determines the final mortality in case of the S flies but at least 12 hours at 15° are needed to have even a slight influence upon the final mortality with B flies. Conversely, 12 hours at 35° had no effect upon final mortality with S flies but strongly influenced that of B flies. A practical conclusion is that susceptible flies are more imperiled by exposure to DDT during comparatively short periods at lower temperature such as those during the hours of night than are resistant flies.

The absorption of DDT by adult flies from oil solution increased as the temperature rose but it was less influenced than was absorption from dry deposits. The initial differences in total absorption at 35° and at 25° disappeared within 24 hours and total absorption by 48 hours was the same at 35°, 25° or 15°. The absorbed but unreacted DDT in S flies exposed to oil solutions at 35° was high within 2 hours and very slowly decreased with time. The unreacted DDT in B flies similarly exposed at 35° or 25° was much lower and remained practically unchanged for 48 hours. The rate of DDT metabolism was especially important in the first few hours. With the two strains of flies the per cent absorbed DDT metabolized was: at 1 hour, S 3.6%, B 48%; at 2 hours, S 15%, B 67%; at 4 hours, S 23%, B 74%, all at 35° C. The high percentages in case of the resistant flies answer the criticism that metabolism is too slow to have an effect upon mortality. Calculation by time intervals shows that after a few hours the temperature coefficient (for 35°/15° C) of DDT metabolism steadily increased while that of absorption decreased. Thus if an insect lived for a few hours at the higher temperature it had an increasing chance of surviving. Survival is favored by increased temperature whenever conditions of exposure are such that after a short interval the rate of detoxification or excretion exceeds that of intake. This holds for single exposures unless they are so massive that the insect dies within a few hours. Such heavy doses always have a positive temperature coefficient of mortality.

The log dosage-probit lines for each strain of flies were parallel at the three temperatures. Thus there was no effect of temperature upon the heterogeneity of the strains in their reaction to DDT. The resistant strain had much flatter ld-p lines than the susceptible strain and hence was more heterogeneous in its response.

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Effect of Timing of Oil Spray Applications During the Fall on Juice Quality and Yield of Lemons in Two Orchards in Southern California[1]

L. A. RIEHL, L. R. JEPPSON, and R. T. WEDDING,[2] *University of California Citrus Experiment Station, Riverside*

Because of information reported for experiments with oranges, California lemon growers have been concerned about the deleterious effects of oil spray, applied for pest control, on the juice quality and yield of lemons. Yothers & McBride (1929), in Florida, and Sinclair *et al.* (1941), in California, found that oranges from trees fumigated with hydrogen cyanide, or left untreated, generally contained higher percentages of total soluble solids than comparable fruits from trees treated with petroleum oil spray. As a result of resistance of scale insects (Quayle 1941) and changes in various economic factors, fumigation with HCN has been largely discontinued as a commercial practice in California. Oil spray for pest control has been used extensively on citrus in California for many years; specified standards for the refinement and grades of spray oils were adopted in California in 1932 (Erb 1932, Marshall 1932).

Newcomb (1947) compared the effects of oil spray and of fumigation with HCN for the control of scale insect and mite pests on lemons in the vicinity of Corona and concluded that oil spray was the pest control treatment of choice. The data for yield did not show any difference between the two treatments. However, Newcomb pointed out that timing of the spray treatments was an important factor in avoiding such damage as leaf drop, fruit drop, inhibition of degreening (Winston 1931), sunburn of fruit rind and leaves, and the discoloration of the fruit rind known as "bronzing," which is related to the occurrence

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The pesticide chemicals discussed may not be used unless a tolerance has been established or an exemption from the requirement of a tolerance has been granted for each specific use, irrespective of the information contained in this report.

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of temperatures below freezing within a few days after spray application.

The relation of application timing to juice quality of oranges from trees sprayed with oil in Florida was studied by Thompson & Sites (1945). In California, the effects on Valencia oranges of single applications of oil spray annually, in each of the different months of the year, have been studied by Riehl *et al.* (1956). The effects on juice quality and yield produced a relatively smooth curve, with the highest value for the application in August or September and the lowest value for the application in April.

Experiments conducted by Jeppson *et al.* (1956) to evaluate the relation of spray application timing to citrus red mite control offered the opportunity to determine the effects of single applications of oil spray annually to lemon trees, in any of the months from August through January, on the juice quality and yield of the fruit.

MATERIALS AND METHODS.—Experimental blocks were established in two lemon orchards; one at the Irvine ranch near Tustin in Orange County and the other at the Los Posas ranch near Somis in Ventura County. Each of the orchards had been sprayed twice annually, fall and spring, with California light-medium-grade spray oil (Erb 1932, Marshall 1932) at 1.75% in aqueous mixture; the last treatment at Los Posas was on February 17, and the last one at Irvine was on April 17, 1952.

The Irvine orchard was composed of 5-year-old trees of Rosenberger Lisbon lemon on sweet orange rootstock. The experimental block was 18 trees long by 10 trees wide. The Los Posas orchard was composed of 4-year-old trees of Eureka lemon on sweet orange rootstock. The shape of the experimental block was roughly similar to that of a right-angled triangle with sides 8 and 34 trees long, respectively; the longer dimension was in the direction of the irrigation. The series of treatments and the basic experimental design were the same in each orchard. Each plot consisted of 4 trees in a square, and each treatment was replicated 3 times.

Tillage, irrigation, fertilization, pruning, picking dates, and other factors of orchard care and management practiced by the growers were not altered for the experimental blocks. Although these practices varied in the two orchards, the treatments of each experimental block were comparable.

The oil used was a proprietary California light-medium-grade emulsive spray oil having the following properties: average molecular weight, 284; unsulfonated residue, 92.5%; viscosity S.S.U. at 100° F., 67.0; composition by weight: 2.4% aromatic carbon, 50.6% naphthenic carbon, and 47.0% paraffinic carbon; fractional distillation: 10% at 570° F., 50% at 623° F., 60% at 636° F., and 90% at 685° F. The oil was used in dilute aqueous emulsion at the rate of 1.75% (7 quarts per 100 gallons of spray mixture).

Each plot was treated twice annually, once with oil spray and once with ovex at the rate of 1 pound of the 50% ovex wettable-powder formulation per 100 gallons of spray mixture. Treatments began in August, 1952, and continued through July, 1955. Plots treated according to the following schedules were selected for determinations of juice quality and yield.

	Month of Application	
Treatment No.	*Oil Spray*	*Ovex*
1	Untreated	Untreated
2	August	February
3	August	March
4	September	March
5	October	March
6	October	April
7	November	May
8	December	June
9	January	July

Applications were made during the first week of the month indicated. The orchard spray equipment was the conventional type, utilizing a high-pressure reciprocating-type pump operating at 550 pounds pressure and provided with satisfactory agitation in the spray tank. The spray guns were operated manually. The applications were made as full-coverage sprays, and a sufficient volume of material was applied to each tree to wet the foliage to the point of drip.

Fruit Sampling and Juice Analysis.—Fruits for juice-quality determinations were taken at random around the periphery of the tree, within a zone 3 to 8 feet above the ground. A sample of 5 fruits taken from each tree was pooled to make a sample of 20 fruits for each plot. When lemons are picked commercially, it is customary to take only fruit that is larger in equatorial diameter than a specified ring size. In the packing house the lemons are segregated into several classes based on rind color (Bartholomew & Sinclair 1951). For juice-quality determinations, the "silver" class provides fruit in a definite stage of maturity which has easily recognized limits. For our purposes, "silver" fruits were defined as those in which both yellow and green were present in the rind in any proportions.

In southern California lemon orchards, the fruit may be picked as often as each month. The periods of winter, spring, and late summer were selected for the collection of samples for juice-quality determinations, and collection always preceded any spray application scheduled. Each time samples were collected, the proper sizing-ring was used, and only fruits in the "silver" class and of the specified size were taken; therefore, results for the various treatments are comparable for each set of samples. Samples were collected at the Irvine ranch in February, April, and September, 1953; in February, 1954; and in January, April, and October, 1955. Those at Los Posas were taken in January and April, 1953; in January and September, 1954; and in January, April, and October, 1955.

The fruit samples were brought to the laboratory for processing. Juice was obtained by reaming the fruit manually on a small power-driven machine. Determinations of juice quality were made in accordance with the methods of analysis used by Sinclair *et al.* (1941). The percentages of total soluble solids, acid and juice were determined, and from these were calculated the pounds of soluble solids per ton of fruit, the pounds of acid per ton of fruit, and the ratio of soluble solids to acid.

Fruit Yield.—Records of yield were taken in 1955 during the third successive year of the treatment program. The program of picking dates and the type, color classes, and sizes of fruit picked were determined by the packing house; data on yield were taken each time the orchard was picked during 1955. For the yield records, each tree

was picked as a separate unit and the fruits from that tree were put into a box placed beside the tree for that purpose. When the pick had been completed for a given tree, the net weight in pounds of fruit per tree was determined. In the course of each pick the mean net weight in pounds of fruit per field box was determined for the type of fruits taken for the given pick; this value could then be used to express the yield of each tree in terms of field boxes. After completion of the last pick in 1955, the data for each tree were pooled to give the total yield per tree for the year.

DISCUSSION OF RESULTS.—Fourteen sets of samples for juice-quality determinations were taken from the two orchards during the experiment. Analyses of variance were calculated for each of six juice-quality factors for each sampling date for each experimental block. At odds of 19:1, differences between treatments were not significant in 81 of the 84 separate analyses; as exceptions, significant differences occurred once in pounds of acid per ton of fruit and twice in ratio of soluble solids to acid. Such exceptions could be expected in random samples particularly as none of the mean differences were significant at odds of 99:1.

Analyses of variance of the data of each experimental block for total yield for the year 1955 showed that the differences between treatments occurred by chance.

Both of the orchards used are located in districts in which climatic conditions are generally moderate. These circumstances facilitated comparisons with respect to the effects of oil-spray timing on juice quality and yield. The results obtained should not be interpreted to mean that qualifications for timing, such as those pointed out by Newcomb (1947) for the Corona district, or similar established practices related to specific locality climatic conditions of other districts, can be disregarded. However, we many conclude that when proper practices for oil spray are followed and the application has been timed to avoid predicted unfavorable weather for several days after treatment, the likelihood is good that oil spray in itself will not reduce juice quality or yield of lemons in southern California, and that the month of application for the period of August through January is not critical to the effect.

SUMMARY AND CONCLUSIONS.—The effect of single annual applications of oil spray to lemon trees, in any of the months from August through January, on the juice quality and yield of lemons in two southern California orchards was studied for the seasons of 1952 through 1955. Within the conditions of the experiments, the evidence supports the conclusion that differences in juice quality and in yield between the treatments were not real and consistent but were within the limits of natural variation.

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Mass Rearing of Six-Spotted Leafhoppers[1]

R. G. STRONG[2] and W. A. RAWLINS, *Cornell University, Ithaca, New York*

Transmission of aster-yellows virus to lettuce by the six-spotted leafhopper, *Macrosteles fascifrons* (Stål), may be reduced to a low level by control of the leafhopper. Evaluation of an insecticide program is based usually on incidence of disease rather than reduction of leafhopper populations. This is due to the difficulty of estimating populations in the field. The insects are rather active, and in a low growing crop such as lettuce collection methods are inadequate to determine accurately the populations therein.

Relying on disease incidence as the sole criterion for evaluating the control program leaves much to be desired. It is desirable to determine the effectiveness of an insecticide in reducing leafhopper populations by direct contact to the insects. In addition, it is important to evaluate the protection provided by the insecticide coating on the plants. Undoubtedly, a certain proportion of the insects in the field at the time of application escape being hit by the insecticide. Also, leafhoppers migrate to the lettuce planting from nearby breeding areas. Consequently, the effectiveness of the insecticide coating is an important aspect of the program.

To evaluate this point, a close study of caged plants was necessary. Leafhoppers were needed in abundance and the ordinary breeding areas could not be depended

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[2] Present address: Department of Entomology, University of California, Citrus Experiment Station, Riverside, California.

FIG. 1.—General view of six-spotted leafhopper rearing set-up in greenhouse. Cages used for insect cultures, at left; transfer-box for adult leafhoppers, at right.

upon for adequate supplies. Large populations had to be reared to carry on the experiments.

During the colder seasons, from September through May, leafhoppers were reared in a greenhouse where it was possible to control temperatures within the range of 70° to 80° F. except on occasional bright days when temperatures would reach 90° F. for short periods. Insectaries were used for summer rearing, from June through August, which maintained conditions approximating the natural habitat of leafhoppers.

GREENHOUSE PROCEDURES.—*Cages and Food Plants.*—Circular cages 7.5 inches in diameter and 20 inches high which fitted inside the rim of 8-inch flower pots were used as shown in figure 1. Cage frames were constructed from 1-inch strips of cellulose acetate glued together with acetone. Nylon tulle with 24×34 threads per square inch, or nylon marquisette containing 27×34 threads per square inch, made excellent covering for cages and was practically escape-proof for adult leafhoppers. The material was fastened to the side of the frames by applying a small amount of acetone. A removable top was made from a separate piece of cloth held in place with a hoop of cellulose acetate. An insect transfer opening was provided for each cage by cutting a 1-inch diameter hole in the center of a 2-× 2-inch piece of cellulose acetate. This was glued about midway to the side of the cage, and, after clipping the cloth, the hole was plugged with a cork stopper. Similar cages only 10 inches high were occasionally used for rearing.

Erie barley beginning to stool and 1- to 2-months-old, healthy China aster made excellent food plants. However, it was not possible to carry the leafhoppers through a complete cycle on the same plants in the greenhouse. Aphid and powdery mildew attacks, combined with leafhopper nymphal feeding, soon terminated the life of barley plants. Greenhouse whiteflies and spider mites attacked aster plants, resulting in adult leafhoppers becoming stuck to whitefly secretions and nymphs becoming trapped in spider mite webs on the leaves.

Vigorous plants supported 200 to 300 adult leafhoppers per cage very well for as long as 7 days, the maximum time adults were held on the same plants. Excessive numbers caused wilting, discoloration, and other symptoms of injury, but prompt removal of the insects usually permitted plants to recover.

Insect Transfer Methods.—Adult leafhoppers were transferred to fresh plants every 4 to 7 days, depending on the time available. Such regular transfers made it

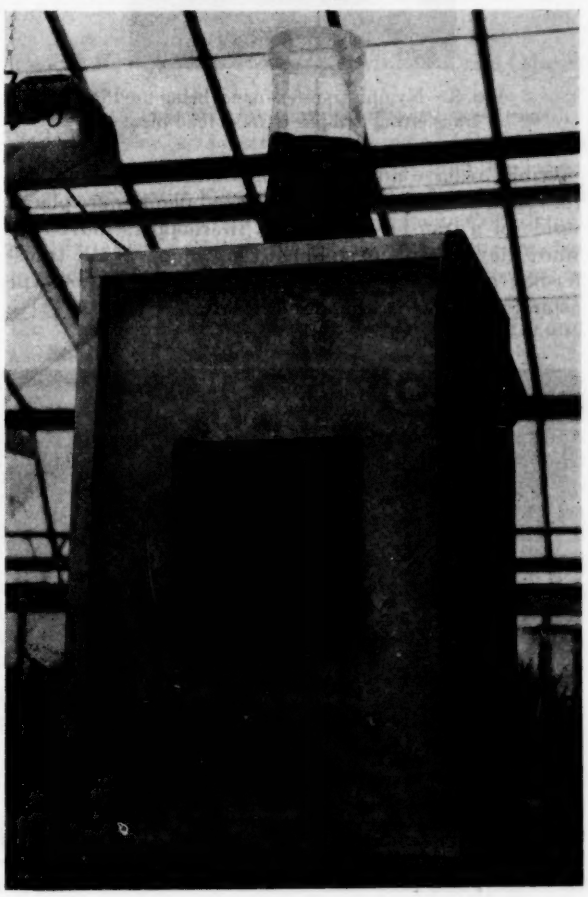

FIG. 2.—Adjustments made to transfer-box for quick transfers of adult leafhoppers.

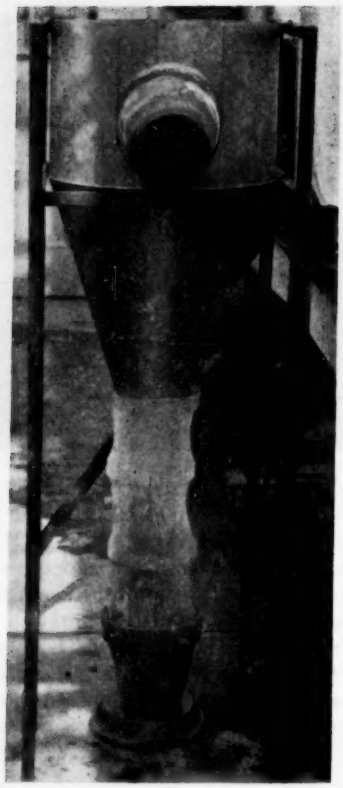

FIG. 3.—Nymph transfer-funnel being used for transferring nymphs to fresh food plants.

possible to have adults of approximately the same age for experimental use. Insects on caged plants were placed inside of a transfer-box (fig. 1). Leafhoppers moved toward the light coming through the window of the transfer-box and adults were collected in a glass-tube-type aspirator similar to that used by Kunkel (1926). These were placed in cages containing fresh food plants.

Occasionally, when time was limited, a special adult-transfer technique was followed by making adjustments to the transfer-box as shown in figure 2. For such emergency transfers, the window on the box was covered, and the lid over a circular opening in the top was removed. An empty cage was set over this circular opening and covered about half-way with black cloth. Insects to be transferred were placed inside the transfer-box. When released they flew toward the light, accumulating in the cage on top. After most of the leafhoppers had moved from their food plants, the cage was quickly placed over fresh plants and the black cloth removed. The few remaining insects were transferred with an aspirator.

One or more transfers of nymphs to fresh plants was necessary. Several methods were tried, but the use of a nymph-transfer-funnel as shown in figure 3 was found to be the most successful for making rapid transfers without losses during the process. Potted plants containing nymphs were placed in the specially designed slot at the top of the funnel. Leafhopper nymphs were then easily brushed or blown onto the fresh plants underneath.

When plants containing a good supply of eggs succumbed, they were placed adjacent to vigorous plants so nymphs could readily move to a food supply. The nymphs were transferred to caged plants after most of the eggs hatched.

INSECTARY PROCEDURES.—Insectary units for rearing leafhoppers and testing insecticides were built near the summer field operations. Temperatures in these were about the same as those found under natural conditions for leafhoppers.

The simplicity of construction of the insectary units is shown in figure 4. Two of the insectaries had tops made from clear vinyl plastic laid over 1-inch-mesh-wire fencing. On the third, the top was made of cellulose-acetate window material, $\frac{1}{4}$-mesh cord embedded in plastic. Removable sides covered with the latter prevented rain and wind damage to the culture. The "floor" of each insectary-

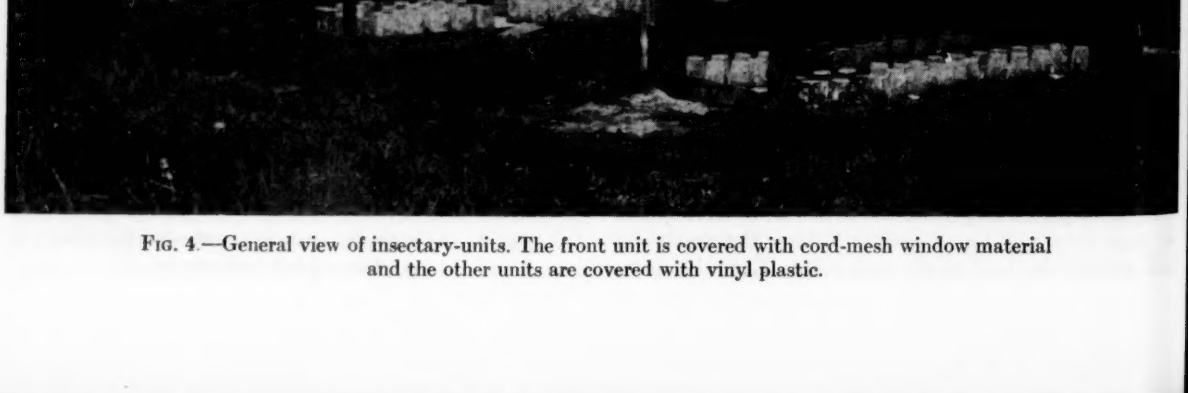

FIG. 4.—General view of insectary-units. The front unit is covered with cord-mesh window material and the other units are covered with vinyl plastic.

unit was made by digging a pit and filling it with peat which was kept wet, thus providing a steady supply of water to plants placed in or on the peat.

The cord-mesh window material appeared better suited for insectary use than the vinyl plastic as it was more durable under the existing temperature changes. The vinyl plastic showed a tendency to tear under conditions of low temperatures and high winds which could result in loss of insect cultures during stormy weather. However, even the cord-mesh material had to be replaced annually since it could not withstand the weight of snow over winter.

All greenhouse rearing procedures were successfully followed in the insectaries. Plants grew more vigorously under the outdoor conditions and fewer transfers were necessary for leafhopper nymphs. Often leafhoppers were carried through all stages of their life cycle, from egg to adult, on both China aster and barley plants.

SUMMARY.—In order to conduct detailed experiments on the prevention of lettuce-yellows by controlling the six-spotted leafhopper, *Macrosteles fascifrons* (Stål), it was necessary to develop mass rearing methods which would supply large numbers of leafhoppers continuously. Procedures are described which are rapid and do not require expensive equipment.

Cages made with cellulose acetate frames covered with nylon tulle or marquisette satisfactorily confined adult leafhoppers. Barley and China aster were used for food plants, and pest problems on these were practically eliminated by alternating food plants for leafhopper nymphs. The use of a transfer-funnel made possible the rapid transfer of nymphs from one caged plant to another. A transfer-box for adult leafhoppers which can be used for detailed transfers, or slightly modified for rapid general transfers, is shown. An account is given of the use of simple insectary-units for summer rearing which approximate natural conditions.

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The Effect of Malathion and Its Isomer on Carbohydrate Metabolism of the Mouse, Cockroach and House Fly[1]

R. D. O'BRIEN, *Science Service Laboratory, London, Ontario*

The insecticide malathion (0,0-dimethyl S-(1,2-dicarboethoxyethyl phosphorodithioate)) is of particular interest for its low toxicity to mammals, and information about its comparative effects against insect and mammalian enzymes may help in explaining this selective action. In a previous paper (O'Brien 1956) it was shown that malathion may have its toxic effect against insects by some property other than anticholinesterase activity. Because the symptomatology of its poisoning of insects involved nerve effects, but cholinesterase seemed not to be the susceptible enzyme, it was suggested in that paper that there might be interference with carbohydrate metabolism. Succinoxidase was eliminated as being insufficiently sensitive *in vivo* and *in vitro* to inhibition by malathion.

The effect of malathion and isomalathion on some enzymes involved in carbohydrate metabolism are reported herein. The enzymes of the hexose monophosphate oxidation system (the 'shunt') which have recently been demonstrated in the house fly (Chefurka 1955), were not investigated. Where possible, it was considered desirable to study whole systems, for example glycolysis, or the total oxidation of pyruvate. The inhibition of any one of the steps in such a system is revealed by an inhibition of the overall reaction, and thus if a compound is ineffective against the whole system, the individual steps do not have to be studied.

Isomalathion (O,S-dimethyl S-(1,2-dicarboethoxyethyl phosphorodithiolate)) has also been studied, because its behavior will probably be similar to that of malathion's oxidation product, "malaoxon" (0,0-dimethyl S-(1,2-dicarboethoxyethyl phosphorothiolate)). Malaoxon is probably a primary metabolic product of malathion metabolism in insects and mammals, and may entirely account for the toxic effect of malathion *in vivo* (March *et al.* 1956).

METHODS.—Where an inhibitor was used it was included both in the substrate-containing and the endogenously respiring samples. Because the inhibitors were insoluble in water, they were dissolved in 5% ethanol. All the uninhibited systems had a similar amount of ethanol added to them. Results in the aerobic studies are corrected for endogenous respiration. Isomalathion was prepared as previously described (O'Brien 1956). The insects were the American cockroach, *Periplaneta americana* (L.) and the house fly, *Musca domestica* L.

GLYCOLYSIS.—*Mouse.*—The system of LePage (1948) was used, with 0.5 ml. of a 10% homogenate of mouse brain, from Swiss albino mice, about 20 gm. live weight, which had been killed by breaking the neck. The levels of endogenous lactate and inorganic phosphate were obtained by adding the homogenate to a vial containing trichloracetic acid plus all the components present in the normal system. Lactic acid was determined by the method of LePage (1951) and inorganic phosphorus by the method of Rockstein & Herron (1951) after precipitation by the method of LePage (1951). The incubations in the Warburg were carried out for 50 minutes at 25° C.

Cockroach and house fly.—The LePage co-factors were added except that nicotinamide was omitted. A 10% homogenate of cockroach thoraces or 2-day-old house flies was made in an ice Potter-Elvehjem homogenizer, using the alkaline potassium chloride solution described by LePage. The cockroach thoraces were taken from

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eviscerated adult females, and had coxae and femora attached. The homogenate was centrifuged at 12,000× gravity for 10 minutes and 1 ml. of the supernatant used. Other conditions were identical to those given for mouse brain except incubation was for 1 hour at 38° C, and the homogenate was tipped into the buffer system from the side-arm after gassing, which maximized CO_2 production.

TRICARBOXYLIC ACID CYCLE.—*Mouse.*—Three mouse brains (obtained as above) were homogenized in 18 ml. of a 1:4 mixture of 0.067 M phosphate buffer (pH 7.4) and 0.9% potassium chloride, using an iced Potter-Elvehjem homogenizer with plastic pestle. One ml. of the homogenate (containing about 75 mg. wet weight of tissue) was used with 1 ml. of cofactor solution, 0.25 ml. of substrate and 0.5 ml. of inhibitor in 5% ethanol, the volume being made to 2.75 ml. with water when necessary. The final concentrations were: phosphate buffer pH 7.4, 0.021 M; ATP, 0.0008 M; fumarate 0.0004 M; magnesium chloride, 0.004; nicotinamide 0.002 M; pyruvate 0.002 M. The center well had 0.2 ml. of 10% potassium hydroxide. The Warburg flasks were chilled in ice until placed on the manometers. The atmosphere was air, the bath temperature 38° C. Exactly 10 minutes elapsed between removing a flask from the ice and closing its manometer.

Fumarase was assayed by a method based on that of Scott (1948). A homogenate of six brains in 35 ml. of buffered KCl was used; 3 ml. were mixed with 1.25 ml. of 0.067 M phosphate buffer (pH 7.4) plus 1 ml. of inhibitor (or ethanol) plus 0.25 ml. of 12.8% sodium fumarate. The mixture was incubated at 38° C. and 1 ml. aliquots removed at intervals of 5 minutes, run into 10 ml. of normal HCl, and titrated with 0.02 M $KMnO_4$.

Cockroach.—Cockroach thoraces, as described above, were ground in an iced mortar with 0.9% KCl containing 0.01 M sodium versenate, at 1. ml. per thorax. The mixture was filtered through cheese-cloth and homogenized in an iced Potter-Elvehjem homogenizer for 1 minute. One ml. was used in each flask, with phosphate buffer, pH 7.4, 0.0103 M; $MgCl_2$, 0.0029 M; cytochrome C, 8.7×10^{-6} M; adenosine monophosphate, 8.24×10^{-4} M; triphosphopyridine nucleotide 4.8×10^{-5} M; substrate, 0.0143 or 0.00143 M; inhibitor, 4.32×10^{-4} M; total volume 3.5 ml. Measurements were made at 30° C. Fumarase was assayed as for the mouse, but with a homogenate of one thorax per 4 ml., and at 30° C.

House fly.—Fumarase was assayed as in the mouse, but a 10% homogenate was used and incubated at 30° C.

RESULTS.—GLYCOLYSIS.—For the glycolytic pathway in the mouse the system of LePage (1948) was selected. Unfortunately its use of fluoride eliminates enolase and phosphoenol transphosphorylase (lactic dehydrogenase is included, because the system is fortified with pyruvate to act as a hydrogen acceptor). An attempt was made to use a non-fluorided system, in the hope that ATP-ase, although it would give erroneous results for phosphorus incorporation, would not be sufficiently active to inhibit glycolysis proper; in other attempts the homogenate was centrifuged and the supernatant used, in an attempt to reduce ATP-ase (Meyerhof & Geliazkowa 1947), although this would also reduce the hexokinase concentration (Crane & Sols 1953). In these experiments, however, carbon dioxide and lactate production were poor, and orthophosphate was increased rather than incorporated. The complete LePage system was therefore used and gave satisfactory acid production, lactate production and phosphate incorporation (table 1).

Table 1.—Effect of malathion and isomalathion on mouse-brain glycolysis.

Additions	μ-moles CO_2 Produced	μ-moles Lactate	μ-moles Orthophosphate Remaining
Water	3.81±0.234	1.69±.009	1.87±.044
Water (omitting glucose)	2.24±0.086	1.11±.011	5.97±.672
Alcohol	3.42±0.105	1.61±.007	2.01±.015
Malathion 5.1×10^{-4} M	2.92±0.192	1.63±.072	2.64±.065
Isomalathion 5.1×10^{-4} M	2.47±0.013	1.61±.008	2.90±.187
Alcohol (endogenous results)	—	0.60±.037	6.02±.030

The effects of malathion and isomalathion upon the system are shown in table 1. Alcohol, normally present in the control and the inhibited samples, was observed to have only a small inhibitory effect. All the inhibitors reduced acidogenesis, but even isomalathion, the most effective, only gave 28% inhibition. Isomalathion and (to a lesser extent) malathion both decreased phosphorus incorporation, the former to the extent of 24%. A similar effect would be shown if these inhibitors interfered with the phosphate determination, and although a separate experiment showed that there was no such interference (presumably because the inhibitors and their hydrolysis products were not precipitated by calcium in the LePage phosphorus method) it is desirable to treat the phosphorus figures with some reservations.

Glycolysis in whole house fly homogenates was next studied. Employing the system used for mouse brain, almost negligible acidogenesis rates were found, and lactate synthesis was poor. Chefurka (1954) obtained good lactate synthesis with house flies using a system varying in several respects from that of LePage. In a series of experiments designed to obtain good lactate synthesis with minimal departure from the LePage system (for the sake of comparison of results with those for the mouse), the following alterations were found to be desirable: use of supernatant from high-speed centrifugation of homogenate (thus avoiding ATP-ase; presumably hexokinase is not particulate in this material); omission of nicotinamide from the system; incubation for 1 hour at 38° C. Under these conditions, acidogenesis was small but easily measurable, and lactate synthesis was excellent. However, ATP-ase presumably outstripped phosphate incorporation, as there was a net increase in orthophosphate. The phosphate figures will still demonstrate any variation in phosphate incorporation as they may be looked upon as the balance resulting from the two opposed processes of incorporation and hydrolysis.

Calculations from the results of the experiments given in table 2 show a lactate synthesis of 1098 γ per gm. per hour for the uninhibited system, which agrees remarkably well with the figure given by Chefurka (1954) of 1038, although he was using a very different procedure. The data of table 2 show that isomalathion inhibits acidogenesis by 13%, lactate production by 10%, and phosphate incorporation by an extent which cannot be stated because the figures are only relative. Malathion itself had none of these effects.

Table 2.—Effect of malathion and isomalathion on fly glycolysis.

Additions	μ-moles CO_2 Produced	Lactate	Orthophosphate
Alcohol	3.73± .0385	1.76± .060	9.31± .090
Malathion 4.3×10^{-4} M	3.51± .197	1.73± .031	9.35± .144
Isomalathion 4.3×10^{-4} M	3.26± 0.111	1.65± .010	9.66± .085
Alcohol (endogenous results)	—	0.54± .082	8.39± .065

Table 3 shows the results for glycolysis in the cockroach. The very large discrepancy between acidogenesis and lactate production was observed in a similar preparation by Barron & Tahmisian (1948). It is surprising that endogenous lactate is so high, yet after incubation in the absence of glucose is quite low. Presumably lactate is utilized during the incubation, and thus its level falls unless more is formed by glycolysis. As in the house fly, neither malathion nor the isomer have any striking effects upon the glycolytic system.

TRICARBOXYLIC ACID CYCLE OXIDATIONS.—*Mouse brain.*—To study the effect of malathion upon carboxylic acid cycle oxidation, it was desirable to obtain a system that would oxidize pyruvate completely. Bartley (1953) studied the conditions necessary for this in a mitochondrial preparation from hog-kidney. In the present study, initial lack of a refrigerated centrifuge precluded mitochondrial studies and whole homogenates were used. These should give substantially similar results, and are less removed from *in vivo* conditions, stable, and of higher activity than fractionated materials for many processes.

Using brain homogenates fortified by the co-factors used for mitochondrial preparations by Green *et al.* (1948) or Bartley (1953), no activity could be obtained. Activity was obtained by adding nicotinamide (Mann & Quastel 1941), and it is therefore assumed that the mitochondrial preparations were free of DPN-ase, which must therefore be in the soluble fraction. The ATP level had to be as high as that used by Bartley, but fumarate could not be replaced by bicarbonate as found by that author for kidney mitochondria.

Evidence that an adequate proportion of the pyruvate was being completely oxidized was obtained by showing a satisfactory ratio of oxygen consumed to pyruvate added and considerable inhibition by malonate and fluoride, *viz.* 64% by 0.004 M malonate or by 0.04 M fluoride. Pardee & Potter (1949) showed that this level of malonate produced its effect solely upon succinoxidase. Fluoride at the level used has been found to inhibit succinoxidase by about 60% (Slater & Bonner 1951).

The ratio of total oxygen uptake to pyruvate added would be 2.5 for complete oxidation, and ratios of this order have been obtained by Green *et al.* (1948) and Bartley (1953) with mitochondrial preparations from kidney. The present system gave a ratio of 1.24, which by Bartley's calculation indicates that 64% is being oxidized to acetate (which here and later implies acetate or material at an equal state of oxidation) and 36% to completion; but this calculation assumes that there are only these two possible fates, and in fact much oxidation to carboxylic acid cycle intermediates probably occurs. The ratio is better than that of 1.1 obtained by Larner *et al.* (1949) using whole homogenates of mouse brain under conditions very similar to those used here. Taking this result in conjunction with the malonate and fluoride effects, it may be assumed that a sufficient fraction of the pyruvate is being completely oxidized to assure that any inhibition of carboxylic acid cycle enzyme will be detected by an inhibition of pyruvate oxidation in the system described.

Table 3.—Effect of malathion and isomalathion on roach glycolysis.

Additions	μ-moles CO_2 Produced	Lactate	Orthophosphate
Alcohol	5.35±0.146	0.35±0.035	1.0±0.12
Malathion 4.3×10^{-4} M	5.59±0.116	0.47±0.045	1.1±0.01
Isomalathion 4.3×10^{-4} M	5.30±0.052	0.38±0.056	1.4±0.01
Alcohol (no glucose)	5.43±0.041	0.09±0.047	1.6±0.01
Alcohol (endogenous results)	—	0.36±0.009	1.3±0.12

Both malathion and isomalathion had quite a strong inhibitory effect on pyruvate oxidation. They were therefore tried at concentrations spanning the rather narrow range between maximum solubility and detectable inhibitory concentrations. The results (referred to an alcohol-containing control) are given in figure 1. Alcohol alone, at the same concentration as in the inhibitor solutions, gave 10% inhibition. The data from which these results are calculated were corrected for endogenous respiration by substracting the oxygen uptakes of substrate-free systems containing the appropriate inhibitors. The endogenous respiration is about 37% of the total and therefore errors could be brought in by correcting in this way, as the assumption is made that the endogenous uptake proceeds identically regardless of the presence of the substrate. The assumption is unprovable, and not particularly likely, but is one that can hardly be avoided.

Malathion and isomalathion at 3×10^{-4} M inhibited the pyruvate oxidation system by 50%. Malathion is therefore about 20 times more effective against this system than against serum or erythrocyte cholinesterase *in vitro*, but isomalathion is about 10 times less effective than against these cholinesterases (O'Brien 1956).

The above results suggested that malathion can block one or more systems of the tricarboxylic acid cycle. The effect of a single level (5.6×10^{-4} M) of malathion and of isomalathion upon the oxidations of the principal compounds of the cycle was studied. The results, given in table 4, suggested that the main block was in the oxidation of citrate. Unfortunately this critical citrate oxidation was also the least efficient in the uninhibited system, the oxidation rates and yields being very small.

Table 4.—Effect of malathion and isomalathion upon oxidation of citric-acid cycle intermediates by mouse brain.

Substrate	Final Concn. (mM)	μLs. O_2 per 30 Mins.	Per Cent Inhibition by Malathion 5.6×10^{-4} M	Per Cent Inhibition by Isomalathion 5.6×10^{-4} M
Pyruvate	2.0	59	80	79
Citrate	2.0	9	100	100
Citrate	20	10	100	
Citrate	100	7	100	
Isocitrate	20	1	—	—
Oxalo-succinate	2.0	32	57	17
α-keto-glutarate	2.0	78	31	27
Succinate	2.0	16	−19	−12
Fumarate	2.0	19	77	40
Malate	2.0	24	35	58
Oxalo-acetate	2.0	48	49	41

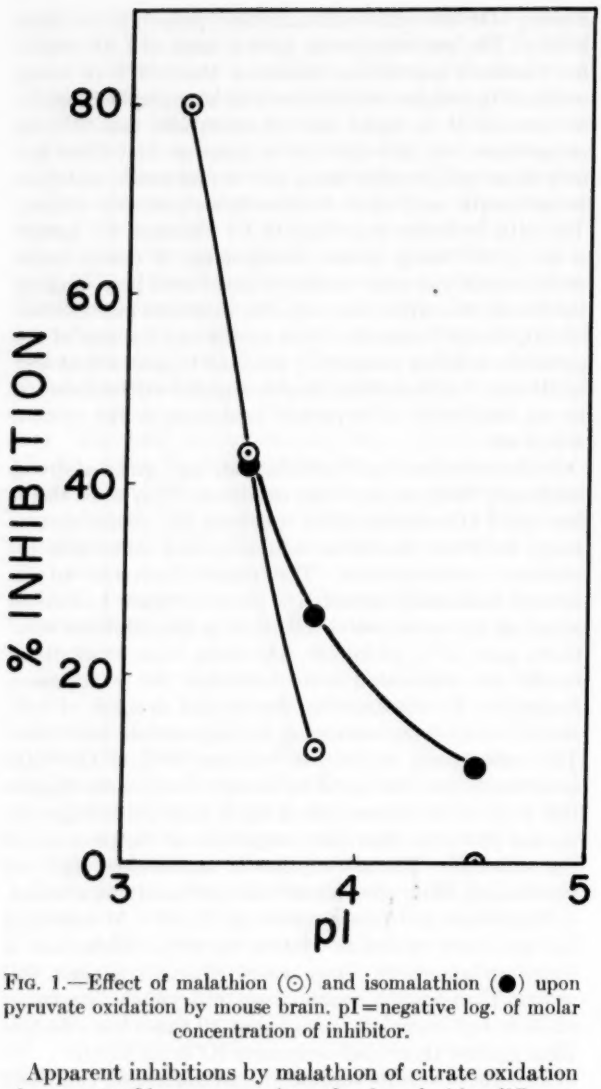

FIG. 1.—Effect of malathion (⊙) and isomalathion (●) upon pyruvate oxidation by mouse brain. pI = negative log. of molar concentration of inhibitor.

Apparent inhibitions by malathion of citrate oxidation of over 100% were consistently found (six different experiments, each in triplicate), *i.e.* after corrections of the citrate-present uptakes by the appropriate endogenous uptakes, negative values were obtained. Separate endogenous controls were always run with and without malathion. The conclusion is that a system containing citrate and malathion has a lower uptake than one with malathion alone. This was confirmed in three experiments in which the oxygen consumption was followed for malathion in treated homogenates before and after tipping in citrate, as shown in figure 2. The scatter of the points in figure 2 reflects the difficulty of accurate measurements where small differences of the order of 5 to 10 μl. were obtained after subtracting large endogenous uptakes of the order of 30 to 70 μl. Errors intrinsic in the manometric procedure are also maximal in this range. For these reasons the effect of varying malathion concentrations was not studied, and the results should be considered as only semiquantitative.

Two possible explanations for the citrate-malathion effect are: (a) the compounds react to give an inhibitor more powerful than malathion, which then inhibits a larger fraction of the endogenous respiration. (b) citrate depresses endogenous respiration, perhaps by complexing essential metal ions. The low oxidation of citrate in uninhibited systems after correcting for endogenous respiration may be a combination of depressed endogenous respiration and good citrate oxidation. If malathion suppressed citrate oxidation, then addition of citrate to a malathion-containing system would result in the endogenous-depressing effect being revealed.

As poor citrate oxidation seems characteristic of brain tissue, the effect of malathion and its isomer upon the vigorous citrate oxidation (70 μl. O_2 in 30 min.) of liver was studied. Malathion gave an apparent negative inhibition of citrate oxidation, perhaps due to oxidation of the inhibitor. However extra uptake due to malathion was 94% of the total required to oxidize it to the phosphate analogue, an unexpectedly high yield. No malathion was oxidized in the citrate-free control. Current studies in this laboratory (unpublished) show that malathion is oxidized by preparations of liver, but not of brain, through a system requiring reduced diphosphopyridine nucleotide (DPNH). The oxidation of citrate presumably provides the necessary DPNH. Isomalathion inhibited citrate oxidation of liver by 6%. This may represent a larger inhibition coupled with stimulation due to oxidation of the unchanged malathion present with the isomer, as discussed below.

The negative inhibitions shown by malathion against succinate oxidation by brain may be due to oxidation of

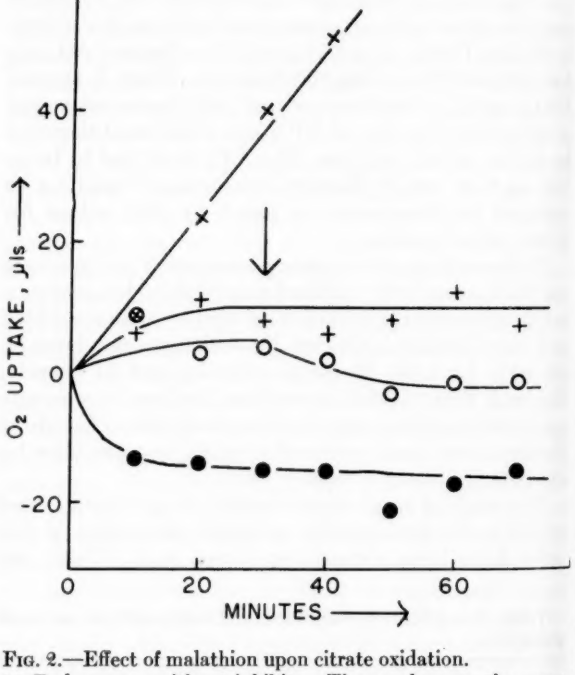

FIG. 2.—Effect of malathion upon citrate oxidation.
× Endogenous, without inhibitor. These values, or the appropriate endogenous controls with inhibitor, were subtracted from the total uptakes to obtain the other curves shown.
+ Citrate oxidation.
⊙ Citrate oxidation, malathion added at arrow.
● Citrate oxidation, malathion added at usual time, i.e., 10 minutes before first measurement.

Table 5.—Effect of malathion and isomalathion upon oxidation of citric-acid cycle intermediates by cockroach thorax.

Substrate	Final Concn. (mM)	µLs. O_2 per 40 Mins.	Per Cent Inhibition by Malathion 4.32×10^{-4} M	Per Cent Inhibition by Isomalathion 4.32×10^{-4} M
Pyruvate	14.3	−43	—	—
	1.43	54	13	0
Citrate	0.143	37	17	9
Isocitrate	1.43	44	46	0
α-keto-glutarate	14.3	45	19	0
Succinate	14.3	149	11	9
Fumarate	14.3	39	20	13
Malate	14.3	21	48	19
Oxalo-acetate	14.3	44	5	0

the malathion by the preparation, using succinate as an energy source, so that the addition of malathion actually increases oxygen consumption. Succinate is not well oxidized and the extra oxygen uptake given by malathion is only a few µls. It is surprising that isomalathion should be oxidized, as the very oxidizable grouping P→S of malathion is lacking as the isomer. However, the very small uptake may be due to the malathion present as impurity in the isomer.

House fly.—A study of the conditions necessary for oxidation of carboxylic acid cycle intermediates by house fly homogenates yielded disappointing results, with small and erratic oxygen uptakes. The techniques of Watanabe & Williams (1951) and Sacktor (1955) for the house fly, and modifications of the methods used for the locust by Rees (1954) and for the bee by Hoskins *et al.* (1956), gave inadequate oxidations, particularly for pyruvate.

Cockroach.—A combination of the methods of Rees (1954) and Hoskins *et al.* (1956) gave satisfactory results for cockroach muscle, when high tissue concentrations were used. The results given in table 5 show generally smaller inhibition than those found for mouse brain particularly for the case of pyruvate oxidation. Citrate did not give the curious effects observed for mouse brain, nor was there any malathion oxidation so substantial as to give stimulation of oxygen uptake. Malathion was uniformly more effective than isomalathion, which in four cases had no inhibitory effect.

FUMARASE.—Fumarate oxidation was greatly inhibited by malathion in mouse brain, and moderately in cockroach thorax (tables 4, 5). The inhibition could be caused by an effect upon the enzymatic hydration by fumarase, or upon subsequent oxidation of the derived malate. Fumarase was therefore assayed in the systems under study, and the effect of the inhibitors upon it was investigated. The results, given in table 6, show generally small effects. The 30% inhibition by malathion of mouse brain fumarase, coupled with the 35% inhibition of malate oxidation (table 5), accounts fairly well for the 77% inhibition observed for fumarate oxidation.

Table 6.—Effect of malathion and its isomer upon fumarase of the mouse, house fly and cockroach.

Source	Activity[a]	Per Cent Inhibition by Malathion	Per Cent Inhibition by Isomalathion
Mouse (brain)	7.96	30	9
House fly (whole)	11.50	20	0
Cockroach (thorax)	6.59	19	13

[a] Expressed in µ-moles fumarate hydrated per hour per gm. wet weight of tissue.

SUMMARY.—Studies have been carried out on the effect of malathion and isomalathion on carbohydrate metabolism in mouse brain, cockroach muscle and whole house fly.

In the brain of the mouse, malathion and isomalathion are effective inhibitors of pyruvate oxidation, perhaps by interference with the citrate oxidizing system. Apart from this inhibition, the glycolytic and tricarboxylic acid cycle pathways of carbohydrate metabolism in the mouse and cockroach (and in the house fly where observations were possible) are not substantially inhibited by these organophosphates *in vitro*. Furthermore, the *in vitro* results show generally smaller inhibitions for insect than for mouse enzymes. Since malathion is far more toxic to insects than to mammals, it is unlikely that the interferences with carbohydrate metabolism *in vitro* have any connection with the insecticidal action of the compound.

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The Evaluation of Triethyl Tin Hydroxide and its Esters as Insecticides[1]

MURRAY S. BLUM and FRANK A. BOWER, *Chemicals and Plastics Division, Quartermaster Research and Development Center, Natick, Massachusetts*

The toxic properties of organotin compounds were first described by White (1886), who found that triethyl tin acetate, in contrast to inorganic tin salts, was highly toxic to dogs, rabbits and frogs. Kerk & Luitjen (1954) reported a similar effect in studies of the fungicidal activity of certain heavy metal compounds. Mercury, copper, zinc and cadmium were found to be fungicidal in the ionic state while ionic tin was non-toxic to fungi. Certain organotin compounds, however, were found to be highly toxic. This property of being essentially non-toxic in the ionic state and highly toxic in the non-ionic form, gives tin a unique place among the heavy metals.

Until recently, possible uses for organotin compounds had received little consideration. The use of tetra-alkyl and tetra-aryl tin compounds as moth-proofing agents was claimed in a patent granted to I. G. Farbenindustrie A.-G. (1929a). Later patents (I. G. Farbenindustie 1929b, Hartmann *et al.* 1930a, 1930b) claimed as effective moth-proofing agents all organic compounds of quadrivalent tin having one atom of tin per molecule. A more recent patent (N. V. de Bataafsche Petroleum Maatschappij 1943) claimed the use of triethyl tin chlorides in insect sprays.

In this laboratory, triethyl tin hydroxide was found to have a high degree of toxicity to house flies. In order to evaluate further the toxicity of organotin compounds and to study in greater detail some derivatives of triethyl tin hydroxide, a number of esters of triethyl tin hydroxide and various alkyl and aryl organic acids, prepared as part of the elastomer research program at this Center, were studied. Several esters including the triethyl tin ester of chrysanthemum monocarboxylic acid (a hydrolysis product of pyrethrum) and the ester of bis-(*p*-chlorophenyl) acetic acid (a possible degradation product of bis-(*p*-chlorophenyl) methyl carbinol from its metabolism by certain insects) were prepared specifically for this investigation.

METHODS AND MATERIALS.—*Preparation of triethyl tin esters.*—The triethyl tin esters used in this study were prepared by the reaction of triethyl tin hydroxide and the desired acid or its anhydride. The reactions were very rapid, being completed as soon as the reactants were brought into solution. When liquid acids were employed, a solvent was unnecessary. Warming the mixture of organic acid and triethyl tin hydroxide followed by recrystallization gave good yields of pure crystalline compound. Reactions involving solid acids were carried out in alcoholic solution and the resulting esters were isolated by precipitation with water. Recrystallization from ethanol-water or petroleum ether gave pure samples of these esters. One example of each technique is given in detail below and the remainder are summarized in table 1.

Table 1.—Preparation and properties of triethyl tin esters.

Triethyl Tin Compound	Solvent	Melting Point (° C.)	Analysis		Yield (Per Cent)
			Calculated	Found	
Formate	None	55–57	—[a]	—	76
Acetate	None	130.5–131	—[b]	—	60
Acrylate	Ethanol	111–112	C—39.01 H— 6.50	C—38.83 H— 6.60	100
Benzoate	Ethanol	73–75	C—47.73 H— 6.12	C—47.59 H— 6.42	70
(bis-*p*-Chlorophenyl) acetate	Ethanol	112–113	C—49.45 H— 4.95	C—49.50 H— 5.17	93
d-trans-Chrysantheumate	None	129–129.5	C—51.52 H— 8.05	C—51.23 H— 8.29	100

[a] Previously reported; Cahours (1860).
[b] Previously reported; Nesmeyanov *et al.* (1937).

Triethyl tin acetate.—Triethyl tin hydroxide (0.56 gm., 0.0025 mol.), 3 ml. of acetic anhydride and one drop of pyridine were mixed in a 25 ml. flask fitted with a reflux condenser. The solution was warmed gently to initiate the reaction. After the initial spontaneous reaction had subsided, the mixture was refluxed for 20 minutes and allowed to stand overnight. The white crystalline precipitate was separated by filtration and washed with cold petroleum ether giving 0.40 gm. (60%) of triethyl tin acetate, m.p. 130.5–131.0° C.; lit. m.p. 130–131° C. (Nesmeyanov *et al.* 1937).

Triethyl tin benzoate.—Triethyl tin hydroxide (0.485 gm., 0.0022 mol.) and 0.266 gm. (0.0022 mol.) of benzoic acid were dissolved in 5 ml. of ethanol and heated to boiling. An equal amount of water was added and the mixture cooled. An oil separated which crystallized on standing to give 0.5 gm. of crude triethyl tin benzoate. Recrystallization from cold petroleum ether gave 0.3

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Table 2.—**Toxicity of triethyl tin hydroxide and six of its esters toward DDT-susceptible (S) and resistant (R) house flies.**

Triethyl Tin Compound	Dose (μg.) per Fly	Per Cent Mortality S	Per Cent Mortality R	LD_{50} (μg./fly) S	LD_{50} (μg./fly) R
Hydroxide	0.10	20	10	0.31	0.40
	.25	45	30		
	.50	70	55		
	.75	100	80		
Formate	0.10	35	5	0.25	0.45
	.25	50	25		
	.50	70	55		
	.75	95	90		
Acetate	0.10	15	15	0.30	0.54
	.25	40	25		
	.50	90	45		
	.75	100	75		
Acrylate	0.10	10	—	0.42	0.70
	.25	30	20		
	.50	70	35		
	.75	95	60		
	1.00	—	95		
Benzoate	0.25	10	—	0.52	0.74
	.50	35	20		
	.75	70	55		
	1.00	90	75		
	1.50	—	100		
d-trans Chrysanthemumate	0.25	15	—	0.51	0.93
	.50	45	15		
	.75	75	35		
	1.00	100	55		
	1.50	—	90		
bis(*p*-Chlorophenyl) acetate	0.50	20	—	0.76	1.28
	.75	35	15		
	1.00	65	35		
	1.50	95	60		
	2.00	—	85		

gm. of pure triethyl tin benzoate, m.p. 73–75° C. Anal. Calcd. for $C_{12}H_{20}O_2Sn$: C-47.73; H-6.13. Found: C-47.59; H-6.42.

For the purposes of this study, the various esters and the parent base were evaluated biologically as dilute solutions in redistilled acetone.

House flies used in this investigation were the susceptible Chemical Specialties Manufacturers Association strain and the DDT-resistant Orlando-Beltsville strain. The larvae were reared on moist Purina dog pellets at 30° C.

Twenty adults of both sexes, 3 to 4 days old, were lightly anesthetized with carbon dioxide and treated topically with 1 microliter of a dilute (0.01–0.2%) solution of the compound in acetone. Controls demonstrated that this quantity of acetone was non-toxic to house flies. The solution was administered from a 0.25 ml. tuberculin syringe, the plunger of which was driven with a micrometer. Two duplicates of four replicates at each dosage were run on each compound tested. After treatment, each group of 20 flies was kept in a half-pint Mason jar at 30° C. for 24 hours, at which time a mortality count was made. A suspension of powdered milk was supplied to the flies for this period.

RESULTS AND DISCUSSION.—As the data in table 2 demonstrate, triethyl tin hydroxide and its esters were toxic to susceptible and DDT-resistant strains of house flies. All the compounds were slightly more toxic to the susceptible than to the resistant strain. The LD_{50} values for triethyl tin hydroxide and its six esters for DDT-resistant and susceptible house flies are given in table 2.

Since the parent compound, triethyl tin hydroxide, was at least as toxic as any of the compounds tested, and the esters showed a decreasing toxicity with increasing molecular weight, it would appear that the toxic moiety is the triethyl tin portion of the molecule.

Triethyl tin hydroxide and its esters quickly produced paralysis in house flies. At higher dosages the flies seemed to recover from anesthesia, as evidenced by leg movements, but remained prostrate until death occurred. This phenomenon may have been the result of the overlapping independent actions of carbon dioxide and the insecticide or the result of an interaction between the two compounds. Although no definitive experiments were performed, it is felt that the paralytic effect was due to the organotin compound alone.

Examination of the triethyl tin esters as anticholinesterases demonstrated that these materials do not inhibit this enzyme at concentrations up to 1×10^{-3} molar[2]. However, it was determined that these esters are capable of blocking the spontaneous activity of the isolated central nerve of the American cockroach (*Periplaneta americana* (L.)[3]. Aldridge & Cremer (1955) have shown that triethyl tin sulfate inhibits phosphorylation processes associated with oxidation in the rat brain and liver mitochondria.

These observations support the assumption that the triethyl tin derivatives were responsible for the rapid paralysis observed in the insect. They also offer some insight into their mode of action.

SUMMARY.—The toxicity of triethyl tin hydroxide and its formate, acetate, acrylate, benzoate, bis-(*p*-chlorophenyl) acetate and *d-trans*-chrysanthemumate esters have been investigated. All were found to induce paralysis quickly and to cause death at a very low dosage level. These compounds were somewhat more toxic to susceptible flies than to DDT-resistant flies.

Triethyl tin esters were found to be ineffective as inhibitors of cholinesterase. However, conduction in the isolated nerve cord of the American cockroach was completely blocked by these esters.

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Control of Some Pests of Ornamentals[1]

ERNEST N. CORY and HENRY A. HIGHLAND, *University of Maryland, College Park*

BOXWOOD PSYLLID.—The boxwood psyllid *Psylla buxi* (L.) is an important pest of boxwood. Its distribution is general wherever boxwood is grown. The so-called American and English varieties suffer from its attack. The injury to English boxwood is conspicuous. Injury to the leaves is caused by the feeding of the nymphs during the growing stage of the new foliage. This feeding causes cupping and curling of new leaves, resulting in unsightly distortion of the new foliage.

According to Underhill (1943) the pest overwinters in the nymphal stage. In Maryland feeding begins during the first part of March, and soon after this the typical strands of waxy secretion can be seen. By the latter part of April the nymphs have completed their development and the waxy secretion is conspicuous. At this time some of the nymphs can be seen feeding or resting on the flat surfaces of the new leaves, having left their place of concealment in the leaf axils. The adults begin to emerge during the first part of May, and according to Pyenson (1946) there is an interval of 6 weeks prior to initial oviposition. In Virginia, according to Underhill (1943), large numbers of females can be found depositing eggs from early July into August. The eggs are deposited beneath the bud scales on either terminal or sub-terminal twigs.

TESTS IN 1953.—Malathion and DDT were used in tests against the boxwood psyllid. One hedge of American boxwood, which was 10 feet high, was sprayed on May 11 with 1 quart of 50% malathion emulsifiable concentrate per 100 gallons and a second hedge was sprayed with 1.5 quarts of 30% DDT emulsifiable concentrate per 100 gallons. All emulsions were applied at 250 to 300 lbs. pressure. These treatments were compared on June 11 by counting the psyllids caught in 25 sweeps of each hedge. Table 1 shows the results of these counts.

Hedge 6A and 6B are opposite sides of the same row and is a continuation of the row used as the untreated check. Therefore, the higher counts on 6A and 6B may be a result of the movement of the psyllid from the untreated to the treated portion.

Table 1.—Results of insecticide tests against the boxwood psyllid.

HEDGE NUMBER	TREATMENT	PSYLLIDS PER 25 SWEEPS
1	Malathion	2
2	Malathion	0
3	Malathion	1
4	Malathion	7
5	DDT	6
6A	DDT	41
6B	DDT	62
Check		95
Check		122

Since it was certain that malathion was at least partially effective in controlling this pest and because it is effective in controlling various scale and mite pests of boxwood, all of the bushes, with the exception of one small row of bushes that had been cut back, were sprayed on July 8 with malathion emulsifiable concentrate at the rate of 1 quart per 100 gallons. Pretreatment and post-treatment records were taken of the psyllids caught in 25 sweeps of each hedge. Table 2 shows these results.

Table 2.—Pretreatment and post-treatment counts of boxwood psyllid on sections of hedges sprayed with malathion.

HEDGE NUMBER	PRETREATMENT COUNT	POST-TREATMENT COUNT
1A	22	0
1B		2
2A	12	0
2B		0
3A	18	0
3B		0
4A	66	0
4B		0
5A	22	0
5B		0
6A	212	1
6B		0
7A	213	0
7B		0
8A		0
8B		0
Check		20

Sweeping of these hedges on August 21 resulted in the catch of 4 psyllids in 25 sweeps of the check hedge and 4 psyllids per 25 sweeps each of hedges 6 and 7. No live psyllids were caught on any of the other hedges. Since Hedges 6 and 7 could be sprayed on one side only. this may account for the incomplete kill.

On July 24 a survey of psyllids was made on boxwood on a large estate. This was a very old English boxwood, planted in a formal garden in rows and mazes. Since the plants were very old and had considerable spread, some difficulty was encountered in attaining complete coverage. This boxwood was treated on July 29 with malathion emulsifiable concentrate at the rate of 1 quart per 100 gallons.

A pretreatment survey on July 24 yielded the following numbers of psyllids per 50 sweeps: 32, 197, 33, 241, 120,

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114, 94, 61. Extensive sweeping on July 31 and August 31 failed to catch a single psyllid.

TESTS IN 1954.—Preliminary laboratory tests indicated that malathion would kill the psyllid within the leaf buds before they emerged as adults. On April 21 all of the boxwood on the estate was sprayed with malathion at the rate of 1.5 quarts per 100 gallons. At this time wing buds and long strands of waxy secretion were visible. Six 25-sweep samples on June 21 yielded one live psyllid.

The extensive hedges at the nursery were sprayed with malathion at the rate of 1.5 quarts per 100 gallons on April 29–30. At this time many psyllids were exposed on the flat surfaces of the new leaves.

Treated and untreated twigs having two or more groups of curled leaves were cut and examined under a microscope on May 6. Thirty-three treated twigs yielded 2 live psyllids, while one group of 7 check twigs had an average of 5.7 live psyllids per twig, and another group of 13 untreated twigs had an average of 8 live psyllids per twig. Two 25-sweep samples of unsprayed hedges that had also been unsprayed the previous year yielded 67 and 125 psyllids, respectively. Nine 25-sweep samples of the treated hedges yielded a total of 5 psyllids. There were many curled leaves of this group of hedges, indicating that the spray was applied too late to prevent early curling of the new leaves.

TESTS IN 1955.—On April 28 part of the boxwood on the estate was sprayed with malathion at the rate of 1.5 quarts per 100 gallons. At this time the white waxy secretion was visible. On May 6, 100 treated and 100 untreated curled tips were picked and returned to the laboratory to be run through a Berlese funnel. The funnel ran for 7 days; the treated tips yielded 138 nymphs, the untreated 171 nymphs.

On June 16 all of these hedges were resprayed with malathion at the rate of 1.5 quarts per 100 gallons. Ten 25-sweep samples on May 26 yielded an average of 3.6 psyllids per sample. Twenty 25-sweep samples taken June 7, previous to the second spraying, yielded an average of three psyllids per sample. Five hundred sweeps on July 19 failed to yield a single live specimen.

On June 16 the hedges at the nursery were sprayed with malathion. Because of equipment modification, the malathion was used at the rate of 1.82 quarts per 100 gallons. On May 26, a pretreatment count showed 5.6 psyllids per 25-sweep sample in 7 samples. Twenty 25-sweep pretreatment samples taken on June 6 yielded an average of 5.5 psyllids per sample. Twenty-five 25-sweep posttreatment samples taken on June 22 yielded an average of one psyllid per sample.

Because the writers were unable to recover any live psyllids from the hedges on the estate subsequent to the early treatment in 1954, it was believed that good control had been obtained. In addition to this, microscopic examination demonstrated that the nymphs were killed by early treatment with malathion. Despite this, however, injury to the hedges on the estate was fairly extensive in 1955, indicating the possibility of migration from unsprayed plants.

It is apparent that the adult population can be almost eliminated by the use of malathion. Since Pyenson (1946) has indicated that there is a considerable lapse of time between emergence of the adult and oviposition, it would appear that a spray of malathion in early June should be effective against the adults before they begin to deposit their eggs.

CONTROL OF BOXWOOD LEAF MINER WITH SYSTEMICS.—The Boxwood leaf miner, *Monarthropalpus buxi* (Lab.), is a serious pest of American boxwood. Many methods have been devised and recommended for its control, including fumigation (Smith & Fisher 1930), hot water immersion (Cory & Graham 1930), bait sprays (Cory 1918) and residual insecticides (Pyenson 1946, Schread 1953). Treatment with DDT residues is the most effective of all methods tested, but the timing of applications is very critical. In addition to this, the boxwood mite *Neotetranychus buxi* Garm. may build up large populations on sprayed bushes (Matthysse & Naegele 1952). These control methods are efficient if carried out under ideal conditions, but none seemed to give consistent results.

Preliminary experiments in 1953 using high dosages in soil and foliage treatments in March (results not reported here), have indicated that Systox is effective against the last instar larvae, and that schradan is not effective under these conditions.

Table 3.—Results of treatment of boxwood with systemics for the control of leaf miner.

DATE OF TREATMENT	NUMBER OF PLANTS	TREATMENT	DOSAGE	INFESTATION AS OBSERVED DURING EMERGENCE
1955				
June 1	15	Systox spray	2 pts./100 gal.	None
	5	None		Heavy
	3	Systox spray	2 pts./100 gal.	None
	3	Schradan spray	1 pt./100 gal.	Heavy
	3	Schradan soil	10 ml.	Light to Heavy
	3	Systox soil	37.7 ml.	None
June 23	18	Systox soil	15 or 30 ml.	None
June 21	5	Systox spray	2 pts./100 gal.	None
	8	Systox spray	2 pts./100 gal.	Light
Sept. 1	10	Systox soil	18.8 ml.	None
	10	Systox spray	2 pts./100 gal.	None
	3	None		Heavy

In the tests in 1955, Systox and schradan were used as soil drenches and foliage sprays. In the foliage sprays the bushes were sprayed to the run off point. When applied as a root treatment, the systemic was added to approximately 3 gallons of water and applied to the soil around the trunk of the bush. If necessary clear water was then added until the soil around the base of the plant was thoroughly soaked in order to assure distribution of the material to the roots. The schradan was a formulation consisting of 90% octamethyl pyrophosphoramide; the Systox emulsifiable concentrate contained 26.2% active material. Table 3 gives the results of the experiments conducted in 1955–56, which show that Systox renders the plant toxic to the boxwood leaf miner; schradan was ineffective. The time of application was not critical.

CONTROL OF EUONYMUS SCALE, UNASPIS EUONYMI (COMST.).—This scale insect is frequently found upon various species of the otherwise very desirable groups of shrub and vine euonymus. It attacks several species in such great numbers that the plants become useless as ornamental shrubs. The scales pile up to such a degree that one spraying seldom does a complete cleanup job. The male scales are white and are produced in such

abundance that bushes may appear as though whitewashed.

In Maryland the first crawlers produced by the overwintering females appear during the first part of May. Thereafter crawlers can be found throughout the summer at least until the middle of September, except for a brief period during the first part of July. This observation is based upon field observations and will vary with weather conditions. According to Underhill (1943) the production of nymphs reaches a peak about the last half of May in Virginia, and the second generation young are observed in greatest numbers in August. The females mature sexually in about 6 weeks but do not produce young until they are 10 to 11 weeks old, and produce young for about 4 or 5 weeks.

On April 22, 1953, heavily infested bushes were sprayed with a 1% malathion emulsion. The extremely heavy dosage was used to determine whether or not the malathion would penetrate the scales and kill the overwintering females. The treatment was repeated on May 1. These bushes were examined on June 9 with the following results:

Twig Number	*Live Adult Females*	*Live Nymphs*
1	11	3
2	5	19
3	5	1
4	26	38
(Untreated)		
5	4	
6	59	
7	38	

No attempt was made to count the nymphs on the untreated twig. A 1-inch section of one of the twigs held 102 nymphs, while one leaf picked at random had 248 nymphs on it. Leaves on the treated twigs were practically free of scale.

On June 11 another heavily infested plant was sprayed with malathion at the rate of 1.5 quarts per 100 gallons. Examination a few days later showed that no live crawlers were present, but live crawlers were found in August on this plant.

A group of bushes was treated on July 2 with malathion at the rate of 1.5 quarts per 100 gallons to determine whether or not the new adults would be killed. Examination in August showed a fairly high population of live adults and some crawlers.

On May 20, 1954, a group of 12 bushes ranging up to 10 feet high was sprayed with summer oil 1 quart per 100 gallons plus 30% DDT 1 quart per 100 gallons. Observations on May 31, June 13 and July 27 showed that crawlers were present, though generally in fewer numbers than on unsprayed checks.

At the Bowie Race Course a large plot of euonymus plants was divided into two sub-plots and sprayed with 50% DDT wettable powder 2.5 pounds plus malathion 1 quart per 100 gallons, and the other sub-plot with malathion 1 quart plus summer oil 1 quart per 100 gallons.

On July 27, 10 twigs from each treated plot and 10 from a check plot were examined with the following findings:

Treatment	*No. of Live Scales Present*
DDT and Malathion	0
Malathion and Oil	3-40-1-0-0-0-0-0-0-0
Check	22-10-0-0-0-0-0-0-0-0

On August 16, 10 twigs from each plot were examined under a microscope with the following results:

	Number of Twigs with		
Treatment	*Old Dead Scales*	*Live Young*	*Crawlers*
DDT and Malathion	6	0	0
Malathion and Oil	5	1	0
Check	5	3	5

On March 3, 1955, a large bush was sprayed with a 40% dinitro-ortho-cresol preparation at the rate of 1 pound per 100 gallons. Examination of the bush on May 31 showed that a few nymphs were present, though in much fewer numbers than on the untreated check bush. Dinitro-ortho-cresylate is recommended by Cantelo (not dated).

Dormant control, directed against the overwintering females, is probably effective but should be considered an adjunct method to be used with summer control directed against the crawlers and nymphs. Care must be taken in using dinitro-ortho-cresol because it will stain painted surfaces.

Control of this pest is difficult because of the nearly constant presence of at least a few crawlers throughout the summer. In Maryland recommendations for control should be based upon the following.

1. Application of dinitro-ortho-cresol as a dormant spray in March, before the buds begin to swell. If the bushes are to be cut back, it should be done at this time.

2. Application of malathion or malathion and oil about the third week in May, the first week in June, and the first week in August.

SYSTEMICS FOR CONTROL OF HOLLY LEAF MINER.—The control of holly leaf miner, *Phytomyza ilicis* (Curt.), has heretofore depended upon residual contact sprays. These materials are effective when applied at the right time, but since the periods of leaf miner emergence and the flowering of the tree are somewhat overlapping, there is a danger of killing the pollinating insects which are necessary for the production of berries on *Ilex opaca*. The use of systemic insecticides eliminates the critical time factor and danger to pollinators, it having been shown that demeton (purchased as Systox) is not usually translocated to the nectar and pollen in toxic quantities.

The *Ilex opaca* trees were from approximately 5 to 12 feet in height, most of them heavily infested with *Phytomyza ilicis* (Curt.). Systox was applied as either soil, foliage or trunk treatments. Trunk treatments consisted of a 4- to 6-inch band of 26.2% Systox emulsifiable concentrate or of 90% schradan painted on the trunk just below the lowest branch. Soil treatments consisted of applying approximately 1 gallon of water containing 30 ml. of concentrate to the soil around the base of the tree, then applying clear water to distribute the material to the roots. Foliage treatment consisted of 8 oz. of actual material per 100 gallons applied to the foliage to the run-off point. Table 4 gives the results of these trials. These trees were treated on June 23, 1955, and results observed on April 29, 1956. A very hard rain fell immediately after the treatments were completed.

The Systox soil and trunk treatments were effective, while schradan gave little control except as a soil treatment The results of the foliage sprays are inconclusive because of the rain that fell. However, a previous test

Table 4.—**Results of applications of schradan and Systox to the soil, trunk, and foliage for the control of holly leaf miner.**

NUMBER OF TREES TREATED	TREATMENT	ESTIMATED POST-TREATMENT DEGREE OF INFESTATION
6	Check	Very heavy
6	Systox trunk	Very light
4	Systox soil	None
10	Systox foliage spray	Light
6	Schradan trunk	Very heavy
4	Schradan soil	None

indicates that neither schradan nor Systox is effective when applied as a foliage spray.

A group of eight trees in a commercial nursery were treated with 26.2% Systox emulsifiable concentrate applied to the trunk. These trees were very heavily infested; many leaves each had one or more mines in evidence. Treatment took place on June 13, 1955, and when results were observed at the end of February 1956, the seven remaining treated trees (one had been removed by the owner) were almost completely free of mines. The exception was one small lower branch on one tree. Untreated checks were very heavily infested at this time.

Another test included only a foliage spray, applied on July 10, 1953. The test plots consisted of hedges of holly, trimmed to a height of about 5 feet. Schradan and Systox at a concentration of 0.1% actual material were used. The hedges were examined on April 28, 1954. There were many mined leaves present on the plants that were treated.

Systox and schradan may be used as indicated by the results of the experimental work, but because of the toxicity of systemics, spraying seems generally inadvisable. The trunk treatment is fairly safe if the precautions on the label are followed.

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Control of Root Maggots in Rutabagas, Especially in Muck Soils[1]

A. R. FORBES and K. M. KING,[2,3] *Field Crop Insect Section, Entomology Laboratory, Victoria, British Columbia*

Unsatisfactory control of root maggots in rutabagas (swede turnips) in muck soils has been referred to in earlier papers (King & Forbes 1954, King *et al.* 1955, Forbes & King 1956), which showed that certain treatments with aldrin or heptachlor were highly effective against this pest in mineral soils common in the Pacific northwest. Insecticide companies and others frequently suggest increased dosages to achieve pest control in muck and peat soils. In 1955 experiments were conducted to test the effects in muck soil of higher rates of application of heptachlor, one of the best treatments, in comparison with the standard rate for heptachlor in both muck and mineral soils. In addition, certain modifications of established treatments were tested in mineral soil. Data on the root maggot problem in muck soils from previous years' work are brought together for the first time.

The species involved in these experiments was the cabbage maggot, *Hylemya brassicae* (Bouché). The Laurentian variety of rutabagas was used.

MATERIALS AND METHODS.—In the experiment in muck soil a 4×4 latin square was used with 3 spray treatments and an untreated check. Each plot consisted of 40 feet of rutabaga row.

In the mineral soil, 6 treatments and a check were replicated 5 times in randomized blocks. Each plot consisted of 50 feet of row. For band treatments, dust, granulated, and emulsifiable formulations were compared. Sprays, at a constant total of toxicant per acre, were applied twice at each of 100 and 200 gallons per acre, and once at 200 gallons. The latter was at the time of the first spray application.

Otherwise, procedure was the same in both experiments, as follows: Seeding was done on June 17. The first spray was applied on July 14, just after thinning, and the second on August 10, as late as the use of a power sprayer would have been feasible without injury to the crop. Harvest data were taken on October 18 and 19. both experiments were on one farm.

Soil samples from each experimental site were analyzed by officers of the Field Crops Branch, British Columbia Department of Agriculture. Percentage organic matter was determined by loss on ignition at 450° C. in a muffle furnace.

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[2] Associate Entomologist and Officer-in-Charge, respectively. Forbes is now at the Entomology Laboratory, Canada Department of Agriculture, c/o University of British Columbia, Vancouver 8, B. C.

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Application methods were the same as those used previously (King & Forbes 1954) except that a band treatment was made with emulsifiable concentrate, the toxicant being applied in 200 gallons of water per acre.

Table 1.—Root maggot damage and control with various formulations and methods of application of heptachlor in clay loam soil, Victoria, B. C. 1955.

Method	Formulation	Toxicant per Acre (Pounds)	Mean Damage Index[a]	Control (Per Cent)
Band (dry)	2.5% dust	5	4	95
Band (dry)	2.5% granulated	5	5	93
Band (wet)	emul. conc.	5	4	95
Spray, twice at 200 gal. per acre	emul. conc.	3	1	99
Spray, twice at 100 gal. per acre	emul. conc.	3	8	89
Spray, once at 200 gal. per acre	emul. conc.	3	16	79
Untreated	—	0	75	—
L.S.D. at 5% level			6	
at 1% level			8	

[a] Maximum damage index (King & Forbes 1954), 100.

In the band method, the insecticide was distributed evenly over a 10-inch band centered along the future crop row. The top inch of soil was raked after treatment and the row was then seeded. In the spray method, the application was concentrated on the crowns of the plants and adjacent soil surface.

Formulations and rates and methods of application are shown in tables 1 and 2. Appraisal of the treatments was made at harvest, when the amount of maggot injury on a number of roots from each plot was determined and expressed as a damage index (King & Forbes 1954). Forty roots were taken from each plot in the mineral soil experiment. In the muck soil 20 were taken since germination had been poor in some of the plots.

Results and Discussion.—In mineral soil (clay loam), a preplanting band treatment of 5 pounds of toxicant per acre applied as a dust, granulated material, or emulsifiable concentrate gave highly satisfactory control (table 1). Each formulation gave at least 93% reduction in damage. This extends previous recommendations (Forbes & King 1956) to include the latter two formulations. Two spray applications, each of 1.5 pounds of toxicant per 200 gallons per acre, resulted in 99% control. Applying the same amount of toxicant in two sprays, each at 100 gallons per acre, gave significantly poorer control. A single application of 3 pounds of toxicant per 200 gallons per acre immediately after thinning was markedly less effective than two applications of 1.5 pounds each. The soil in this experiment had an organic matter content of 9% and a pH of 6.0.

In contrast, in muck soil, two spray applications each of 3.5 pounds of heptachlor per 200 gallons per acre gave very unsatisfactory control, only 59% of the roots being marketable (table 2). Similarly, two spray applications at either 1.5 or 2.5 pounds gave poor control, only 42% and 54%, respectively, being marketable. The soil in this experiment had an organic matter content of 49% and a pH of 6.6.

In the previous several years' studies poor control was obtained in muck and in peat soils, as at Alberni in 1951 (table 3), with treatments that were effective in mineral soils. Similar experience was reported to the authors by a number of commercial growers and custom control operators. Thus it was indicated early that soil type influenced effectiveness of control measures.

Table 2.—Root maggot damage and control with heptachlor spray treatment[a] in muck soil, Victoria, B. C. 1955.

Total Toxicant per Acre (Pounds)	Mean Damage Index[b]	Control (Per Cent)
3	47	43
5	36	56
7	31	62
0	82	—
L.S.D. at 5% level	12	

[a] Two applications (July 14 and August 10), each at 200 gallons per acre, with an emulsifiable concentrate containing 2 pounds of toxicant per gallon.
[b] Maximum damage index (King & Forbes 1954), 100.

Table 3.—Root maggot control with heptachlor spray treatments[a] in various soils, Vancouver Island, B. C. 1950–55.

Locality	Year	Soil Data: Classification	Soil Data: Organic Matter (Per Cent)	Per Cent Control: Aldrin	Per Cent Control: Heptachlor
Victoria	1952	loamy sand	9	87	86
Victoria	1954	loamy sand	9	95	95
Victoria	1955	clay loam	9	—	99
Victoria	1953	loamy sand	10	86	93
Courtenay	1951	loamy sand	11	80	89
Courtenay	1953	loamy sand	11	96	99
Courtenay	1954	loamy sand	11	98	99
Courtenay	1952	loamy sand	15	89	86
Courtenay	1950	sandy loam	18	75	58
Alberni	1951	muck	44	45	45
Victoria	1955	muck	49	—	43
Victoria	1953	muck (peat)	59	70	85
Correlation between organic matter and control, 22 pairs: −0.69**					

[a] Aldrin at 5 and heptachlor at 3 pounds of total toxicant per acre (for rows 3 feet apart) in 2 applications.

The 1953 experiment at Victoria gave important quantitative data. One end of this field had received a thin cover of loamy sand (10% organic matter) over the peat soil (59% organic matter). As the experiment was set up, two and one-half replicates were on the loamy sand, and the other two and one-half were on the peat soil. At harvest, damage in the treated plots was twice as great in the peat soil as it was in the loamy sand. This difference was highly significant. Damage in the untreated plots did not differ significantly between the two soil types.

Thus, excellent commercial control of root maggots in rutabagas, though possible in mineral soils in British Columbia as shown here and in previous papers, is not obtained in soils having more than about 20% organic matter. Occasional instances of satisfactory commercial control have been observed in muck soils but these were of relatively low (15 to 20%) organic content, or had received an admixture of mineral soil, or the treatments were at extremely heavy rates of application. In one instance noted by the authors a grower achieved fair com-

mercial control in peat soil (59% organic matter) by combining two recommended treatments each at double the standard rate, i.e., by using per acre 10 pounds of aldrin in a band treatment and a total of 6 pounds of heptachlor in two spray applications.

Future investigation must, therefore, take into account the extreme variability of muck and peat soils. The nature of these soils depends on the depth and characteristics of the original peaty layer, the amount and depth of tillage, and number of years under cultivation.

Recent studies throw some light on the factors that may reduce the effectiveness of insecticide treatments in muck and peat type soils. Cullinan (1949) reported that phytotoxicity of DDT varied in different soil types, being harmless at rates up to 1000 pounds per acre in acid muck soils but injurious at rates as low as 25 pounds per acre in soils low in organic matter. He suggested that low phytotoxicity in soils high in organic matter was due to adsorption. Other pertinent papers dealing with DDT phytotoxicity as affected by soil types are reviewed by Thurston (1953). Fleming & Maines (1953) found that the more organic matter in the soil the less persistent was DDT, being most persistent in sand and least so in muck. The same authors (1954) found that rate of decrease in chlordane residues was related to the amount of organic matter present. The speed of lethal action also showed this relationship (Fleming 1948). These papers suggest that the persistence, phytotoxicity, and insecticidal effectiveness of DDT and chlordane are inversely proportional to the organic matter content of the soil. Some of them further indicate that some process, probably the physical process of adsorption, is involved in causing a loss of biological activity of the insecticide more or less quickly after it is added to the soil.

The authors' results with heptachlor and aldrin spray treatments during several years (table 3) showed a highly significant negative correlation between the percentage of organic matter in the soil and the control obtained. The effect of organic matter in the soil on aldrin and heptachlor may thus be similar to its effect on DDT and chlordane as shown by Fleming & Maines (1953). Poor control obtained with aldrin and heptachlor showed that the loss of biological activity was rapid. Decomposition of insecticide may also take place in these soils but this seems unlikely in view of the known stability of aldrin and heptachlor under a wide range of conditions.

Soil characteristics also modify the behavior of the adult of the cabbage maggot in ways that may influence the control obtainable. The field observations of O'Kane *et al.* (1923, pp. 8–9) showed that the fly tended to insert the ovipositor for egg-laying "between the stem of the [cabbage] plant and the loose, dry, sandy soil"; or, if there was no suitable crevice between the sand and the stem, to find a small cavity in the soil nearby in which to lay her eggs. Egg counts and observations during the 10 years of the present investigations in British Columbia showed that this was the characteristic, though not invariable, procedure. In the heavier-textured mineral soils of most of the experiments, the majority of the eggs were found somewhat away from the stem rather than between it and the soil, usually in very shallow cavities. This tends to increase the chance that the newly hatched maggot will come into contact with a lethal amount of insecticide during its journey from the egg to the plant. In peat soils, and in muck soils of high organic content, however, the surface soil is fluffy and loose, crevices between the stem of the plant and the soil are almost always present, and cavities near the plant are of such size and depth that the fly can easily work its way to a considerable depth before ovipositing. The usual dryness of the surface undoubtedly provides a stimulus toward placement of the eggs at a lower level. Egg counts around plants in loose, friable peat soil in 1955 showed that eggs occurred at deeper levels than in clay loam. Thus, in muck and peat soils some eggs may be laid at levels below those receiving insecticidal treatment, and the maggots hatching from them may reach plants without contacting the insecticide.

Hence it is evident that treatments for root maggots in soil of high organic content may have to extend to a greater depth and use much more insecticide than do those that have been proved effective in mineral soils.

SUMMARY.—Excellent control of the cabbage maggot, *Hylemya brassicae* (Bouché), possible with treatments of aldrin or heptachlor in mineral soils in British Columbia, has usually not been obtained in muck and peat soils. Available data suggest that adsorption of the insecticide by organic matter in the soil and the oviposition habits of the fly in loose soils contribute to the poor control. It seems less likely that breakdown of insecticides is involved.

In mineral soils, band treatments with granulated or emulsifiable formulations give highly satisfactory control similar to that previously shown for dust formulations. For spray treatments, at least two applications are needed. Two hundred gallons per acre per application give better control than 100 gallons per acre per application.

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Biology and Habits of *Pagiocerus fiorii* in Ecuador[1]

HAROLD R. YUST[2]

The most serious pest of stored soft corn in the mountain valleys of Ecuador is *Pagiocerus fiorii* (Egger)[3]. Both the larvae and adults feed within the kernels reducing the corn to powder and hulls. In the warmer valleys, the damage from the weevil is often so severe that the corn has no value within 3 or 4 months. The adults fly to the field and attack the mature corn in the husks so it is usually infested when harvested. This usually amounts to an infestation of only a fraction of 1% of the kernels. Before the days of insecticide treatments the annual loss to farmers was very high. The farmers normally sold their corn as soon as it was harvested which caused depressed prices at that time. Subsequently shortages as well as high prices resulted.

The origin of this pest is not known. It was reported in Los Chillos Valley near Quito in 1940 and later was identified as *P. frontalis* (Eichh). Old residents in various parts of the Andes report that it was a pest many years prior to this date. This seems likely. Wille (1943) states that *P. frontalis*[4] was discovered as a pest of corn in Colombia and Peru in 1930. In Ecuador it is distributed throughout the mountains as reported by Yust & Cevallos (1954–55) from an altitude of 5,000 to 8,600 ft. Since this corresponds to the soft corn growing area, it is doubtful that altitude has much significance. The only reported host in Ecuador is soft corn. It has never been observed attacking flint corn. Wille (1943) reports that it attacks coffee cherries, coffee seeds and seeds of fallen avocado fruit in Peru. This has not been observed in Ecuador although farmers say *P. fiorii* breeds in avocado seed. There is a Scolytid beetle, *Thamnophthorus* sp., (probably unidentified) which attacks the seeds of fallen avocados in Ecuador. It resembles *P. fiorii* so it seems possible that it may be in error to say that avocado seeds are a host in Peru.

This study was undertaken to obtain information on the biology of this pest because of its economic importance to the farmers. No prior studies of any consequence have been made in Ecuador so far as known. In many aspects this study is incomplete because of limitations of time and personnel.

METHODS AND PROCEDURES.—The studies reported herein were conducted during 1955 in Quito primarily in a roof laboratory room which is relatively warm for Quito but probably no warmer than in field grain storage rooms where the beetles are a serious problem. No artificial heat was used and no work was done under controlled temperatures. The maximum and minimum temperatures in the room were recorded for the period from February to August, 1955, and are summarized in table 1. The room was relatively cool but this is typical of grain storage buildings because of the thick masonry walls and tile roofs.

The insects used in this study were obtained from corn storage rooms near Quito in valleys where corn is grown and where the insect is a serious pest of stored corn. Old adults were used at times and were selected at random from infested corn. New adults were obtained from infested corn, brought to Quito, from which the adults emerged. Glass jars and vials were used for cages and rearing containers. Soft corn of a good quality was used for food and oviposition. Corn apparently free of infestation was obtained and heat-treated for 3 hours at about 55° C. to ensure that it was free of eggs or other stages.

Table 1.—Summary of temperatures in the Quito laboratory room.

MONTH, 1955	AVERAGE	TEMPERATURE ° C.			
		Average			
		Maximum	Minimum	Maximum	Minimum
February	17.0	20.6	13.3	27.0	7.8
March	17.3	19.8	14.9	31.1	12.8
April	15.8	18.0	13.5	21.1	12.2
May	17.6	21.5	13.7	25.0	11.7
June	16.7	20.6	12.9	22.8	11.7
July	17.3	21.5	13.2	22.8	11.7
August	17.0	21.6	12.4	23.9	11.1

Groups of 10 beetles were placed in the cages and considered a unit in most of the studies. This procedure was followed because of the uncertainties of the sexes and because of the large amount of work and equipment necessary to handle individuals or pairs. The insects and the host materials were handled as little as possible because preliminary observations showed that the insects did not perform normally when they were disturbed at short intervals.

DEVELOPMENTAL STAGES.—*Eggs.*—The eggs are oval in shape, smooth, bright and pearly white when first deposited. Before hatching they become creamy white. They vary in size from about 0.9 to 1.1 mm. in length and from about 0.6 to 0.9 mm. in width. The females usually deposit the eggs within the kernels of corn after they enter them through holes made by feeding. The eggs are usually found near the embryo when the corn is very dry but when slightly humid they are found throughout the endosperm. When the corn is on the cob, eggs may be deposited at the point of insertion of the kernel in the cob and in small openings between the kernels. This usually occurs only when populations are high.

From general observations it appeared that the average

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[3] Identified in 1953 by Dr. W. H. Anderson, Section of Insect Identification and Parasite Introduction, U. S. Department of Agriculture, Washington, D. C.

[4] In a talk with Dr. Wille in Lima, March 1956, he stated that this beetle was identified many years ago and he believes it to be what is now called *P. fiorii* (Egger).

female deposited about 80 eggs under the conditions of these studies. Usually two or more eggs were deposited in the same place, apparently in the same stage of development, although as many as seven have been found together. It appears that no special egg cells are made within the kernels as the eggs were found in the powder produced by feeding. Where there was a high population, more than one group of eggs could be found in the same kernel. Eggs removed from the kernels showed high mortality.

Larvae.—The larvae are white when they first emerge from the eggs and change to a light cream color as they develop. The head is oval in shape, smaller in diameter than the body and light yellowish brown in color. The body is without pubescence and shiny. The mature larvae are about 1.6 to 1.9 mm. in length and 1 to 1.3 mm. in width. Those feeding in corn with a high moisture content appear to be larger and seem to develop more rapidly than those in very dry grain.

The larvae are always within the kernels, and although there may be only one, as many as 27 have been found in one kernel where the grain was heavily infested. The larvae are voracious feeders forming galleries throughout the endosperm. Frequently, the galleries are near the surface and can be seen through the cuticle. They are similar in appearance to the trail of a serpentine leaf miner. These sub-surface tunnels are often the first external evidence of infestation. When there are many larvae in one kernel the entire interior may be reduced to powder. The larvae are probably responsible for the major damage to corn. The period from emergence of the new adult to the pupa averaged 37.2 days when the eggs and larvae were not disturbed within the kernels. Larvae removed from the kernels showed high mortality.

Pupae.—The pupae in the early stages are milky white and gradually change to a dark yellow color. They are about 2.6 to 2.8 mm. long and 1 to 1.4 mm. wide. Pupation occurs in a cell within the kernel at the end of the larval feeding gallery. Frequently, the pupation cell is just beneath the cuticle and the pupa appears as a dark spot from the exterior. In heavily infested corn as many as 12 pupae have been removed from one kernel. The average duration of the pupal stage was 8 days.

Adults.—The adults are light yellowish brown immediately after pupation and gradually change to dark brown but are without color markings. The elytron has a series of longitudinal ridges on the center of which is a row of short fine hairs. The thorax is densely covered with minute pits and very fine pubescence. The ventral aspect and appendages are lightly covered with a short pubescence. The body is cylindrical, and the head is depressed. The adult measures about 2.7 to 3 mm. in length and 1 to 1.4 mm. in width.

The adults are strong fliers but show little inclination to fly at temperatures below 22° C. They are positively thigmatropic. They were very restless at first in vials containing only 10 kernels. They prefer darkness and are not inclined to leave the grain until overcrowding results. When infested corn is exposed to the direct sun the adults escape from the kernels and fly away. This is probably a reaction to heat. Observations show that the adults feed and deposit eggs in more than one kernel. Copulation was rarely observed and it is concluded that it is normally at night or within the kernels. When there is visible difference in size the larger beetles are usually females.

Several tests were made to determine the duration of the adult stage. In one such test 10 newly emerged adults were placed in each of 10 cages of 100-cc. capacity with 100 kernels of corn. The containers were inspected at the end of each month at which time mortality was determined and the survivors placed on fresh corn. Infrequent observations were made to reduce disturbance to a minimum. In the calculations it was assumed that the dead insects lived only half of the month in which they died. On this basis, the average adult life in days was 60.2 ± 7.5. In a more comprehensive test, 20 groups of 10 newly emerged adults and 20 groups of 10 old adults, selected at random from infested corn, were placed in vials 2 cm. in diameter by 9 cm. in length. Corn was placed in half of the vials, the other half were without food. Only about 10 kernels were in each vial providing a rather abnormal habitat but it was possible to observe dead beetles as soon as they died. The average length of life, the standard errors for the averages, and the chi-square for each group are presented in table 2.

Without food the newly emerged adults lived significantly longer than the old adults. With food the average length of life was about the same but the data for the newly emerged adults were not homogeneous, suggesting that the test is not an adequate measure of the life span of new adults. It is also possible that most of the old adults were relatively newly emerged as they were obtained from heavily infested corn from which many adults were flying.

Table 2.—The average length of life in days for newly emerged and old adults without and with food.

Adult Beetles	Age (Days)			Chi-square
	Average	Minimum	Maximum	
	Without Food			
New	12.0 ± 0.3	2	16	1.3
Old	9.9 ± 0.3	3	16	2.1
	With Food			
New	66.0 ± 2.7	18	102	55.6[a]
Old	67.7 ± 1.7	36	100	3.5

[a] Exceeds the 1% level of significance.

Length of Life Cycle.—The life cycle was determined as the interval from the parent adult to the appearance of F_1 generation adults. Ten old adults taken at random from infested corn were placed in each of 15 glass cages containing corn. The adults were removed in 1 week. The containers were inspected at regular intervals for newly emerged adults and the results recorded. The minimum period from adult to adult averaged 46.4 days in the 15 cages and ranged from 42 to 53 days The data were highly uniform suggesting that 46.4 days is a good estimate of the minimum life cycle. In this test 843 adults emerged from the 15 cages. The average duration for all emerging adults was 56.5 days and the range was 42 to 94 days. The average was obtained by dividing the total days from parent adults to emerging adults by the

total number of emerging adults, assuming the middle of the week as the date of introduction.

LENGTH OF A GENERATION.—The length of a generation was based on the period from the newly emerged adult until all F_1 adults emerged. To obtain these data, 10 newly emerged adults were placed in each of 10 cages containing corn. The adults were held in the respective cages for 1 month, at the end of which period all live adults were transferred to new cages with fresh corn. The procedure was continued until all parent adults were dead which was for 4 months. The date of the emergence of all new adults was recorded. Based on the average period from introduction of the newly emerged parent adults in the first group of cages until all the progeny of all cages emerged, the average length of a generation was 70.8 days. The range was 42 to 150 days.

TOTAL LENGTH OF LIFE.—No large group was observed from eggs to the death of the adults but separate data may be used as a good estimate of the total length of life. In a previous test, 843 adults emerged in an average of 56.5 days. In table 2 the average length of life of 100 new adults was 66 days. The sum of these two figures is 122.5 days which is probably a reasonably good estimate of the average length of life.

PROPORTION OF SEXES.—No reliable external characters were discovered to easily distinguish the sexes with certainty. Hence, five lots of 100 old adults were dissected and the numbers of both sexes recorded. The adults for this purpose were collected at random in heavily infested corn. The results are presented in table 3 and show that 67.8% of the population were females. The data were tested by chi-square and it was found that none of the groups had a ratio differing significantly from the theoretical 2:1. It seems reasonable, therefore, to conclude that the ratio of females to males is 2:1.

PRODUCTION.—The total production of 10 groups of 10 newly emerged adults was obtained by placing each group in glass cages containing 100 kernels of corn. The adults were held in the respective cages for 1 month, at the conclusion of which all live parent adults were transferred to new cages with fresh corn. This procedure was continued for 4 months when all parent adults were dead. Records were then made of the number of emerging adult offspring and the date of emergence. These observations were continued until emergence was completed. Each adult female produced an average of 75 F_1 adults assuming that two-thirds of the parent adults were females. The average production per day for each parent female was 1.245. For the cages with the first month of egg laying the production averaged 1.463 adults per female adult per day, for the second month 1.252, for the third month 0.674 and for the fourth month 0.948.

Table 3.—Number of females and males in five lots of 100 and the Chi-square values for a theoretical population of 2:1.

GROUP	FEMALES	MALES	CHI-SQUARE
1	66	34	0.048
2	68	32	0.048
3	72	28	1.128
4	64	36	0.407
5	69	31	0.121
Total			1.752

Table 4.—Emergence of F_1 generation adults grouped by months after the newly emerged parent adults were placed in cages.

MONTHS	F_1 ADULTS, PER CENT	AVERAGE PER FEMALE PARENT ADULT	
		Both Sexes	Females
Second	55.0	41.25	27.5
Third	25.2	18.90	12.6
Fourth	13.0	9.75	6.5
Fifth	6.8	5.10	3.4
Total		75.00	50.0

The period of emergence was grouped by month from the start of the new parent adults as shown in table 4. It may be seen that 55% of the total F_1 adults emerged during the second month and 80.2% at the end of 3 months. Furthermore, 50% of the new generation had emerged in 57 days. This explains why the average length of a generation was only 70.8 days although the minimum life cycle averaged 46.4 days. Since 5,025 new adults emerged in the test, the data may be considered reliable.

RATE OF POPULATION INCREASE.—The rate of population increase was calculated on the basis of the data in table 4. It may be seen that 75 adults of the F_1 generation emerged within 5 months after the parent female had emerged. Before all of the first generation had emerged, however, many of the F_2 generation were emerging. These data indicate that about 1200 adults emerged from one parent female in 4 months, about 2,250 emerged in 5 months, about 35,000 emerged in 6 months and about 78,000 emerged within 7 months. If there are 70,000 kernels in 100 lbs. of corn as has been estimated and there is only 0.1% of the kernels with one adult that has just entered, it is highly probable that there will be 4,200 adults in the 100 lbs. of corn at the end of 3 months.

DAMAGE TO GRAIN.—Grain attacked by this beetle is reduced to powder when the population is high. Before this happens the market value of the grain is substantially reduced. It is, therefore, important to know the damage inflicted by the beetles. Attacked grain is also damaged by heating and some information was obtained on this subject.

Number of Attacked Kernels.—In preliminary tests it was found that little could be learned by daily or weekly inspection of containers with a few kernels probably because this does not provide a normal habitat and the insects do not like frequent disturbance. Tests were then made in which 10 new adults were placed in 10 cages with 100 kernels in each. The insects were removed at monthly intervals and placed in cages with fresh corn until all were dead. The grain was examined after the adults were removed and the number of attacked kernels was recorded. The total live-adult-days were calculated assuming that the adults died midway during the month in which they were found dead. The results are presented in table 5. In this study, 620 kernels were attacked by 100 adults during their entire adult life. On the average each insect attacked 6.2 kernels or an average of 6.2% by

Table 5.—Attack of kernels by 100 adults during entire adult life.

Months	No. of Kernels Attacked	
	Total	Average per Insect per 10 Days
1	326	1.15
2	211	1.08
3	62	0.66
4	21	0.67
Total	620	

each adult. Of course, it must be noted that the surviving adults were arbitrarily moved which may have increased the number of attacked kernels, but during the first month alone more than half of the attack occurred. The rate of attack decreased during the first 3 months of adult life as shown by the number of kernels attacked by one insect per 10 days.

Another test was made to determine the number of attacked kernels by a given number of adults and their progeny. Ten old adults were placed in each of 10 cages with 350 kernels in each. Old adults were used because this was considered more typical of what might occur in a storage bin. The parent adults together with their progeny were held in the same cages for nearly 5 months. At intervals all of the kernels were examined and the number of those attacked was recorded as summarized in table 6. The data show clearly the effect of population increase on the damage to corn. In the first 20 days the adults attacked kernels at the rate of about 1.45 per adult per 10 days assuming none died. This is higher than the rate during the first month in table 5. At the time when the second inspection was made, which was at the beginning of the emergence of the F_1 generation, 15% of the kernels were attacked.

The increase in the damage to corn in storage is shown by observations that were made in typical shelled corn and corn on the cob from the field. The corn had been harvested several months earlier and had been stored on the cob. It was from a very cold valley and had been graded once by hand at which time all ears showing appreciable attack were removed from the bin. Some of the corn was shelled for the test. A 100-lb. sack of shelled corn and a 60-lb. sack of corn on the cob were brought to the laboratory where they were stored for about 9 months. At irregular intervals 10 samples of 50 kernels each, taken at random, were examined for insect attack. In the first

Table 6.—Attack of kernels by 100 adults and their progeny.

Period (Days)	No. of Kernels Attacked		Attacked Kernels (Per Cent)
	Total	Average per Parent Adult	
20	289	2.9	8.3
48	524	5.2	15.0
79	1083	10.8	30.9
109	2117	21.2	60.5
140	2493	24.9	71.2

Table 7.—Average increase in damage to soft corn by *P. fiorii* during about 8.5 months.

Inspection Time (Days)	Attacked Kernels (Per Cent)	
	Shelled Corn	Corn on Cob
0	0.8	0.4
39	1.6	2.8
71	5.1	6.5
105	21.0	16.1
134	66.9	44.0
164	87.5	67.4
195	97.5	80.1
225	99.4	99.0
257	100	100

two inspections the samples were taken only from the top of the sacks. In subsequent inspections similar samples were taken from both top and middle and the counts averaged. In the sampling of corn on the cob each unit of 50 kernels was from three ears taken at random. The kernels were not removed from the cobs. The results are given in table 7.

At the time of the first inspection it may be seen that the corn was already lightly attacked. At the time of the third inspection, the damage in the middle of the sacks of shelled corn was 6.6% compared with 3.6% on the top. The fourth inspection showed 34.6% damage in the middle compared with 7.4% on the top. There was enough mixing resulting from the first two inspections that subsequent differences had no meaning, but it may be concluded that deep in shelled corn the insect density is higher than on the surface. In the corn on the cob there was no significant difference in damage in the two locations. There was significantly more damage in the shelled corn than in the corn on the cob in the fifth, sixth, and seventh inspections. This may have been due to the fact that the entire kernel could not be examined while on the cob and some of the damage was therefore probably overlooked.

Heating of Infested Corn.—Temperatures were determined in the middle of the sack of infested shelled corn and in a sack of treated corn containing no infestation. The readings began the fourth month after the corn was brought to the laboratory and were made during half or more days throughout the entire month. The results of these readings are summarized in table 8. The grain was heating badly in the fourth month when about one-third of the corn was attacked. The heating increased through

Table 8.—The average monthly temperature in noninfested and infested corn with associated percentages of attacked corn.

Month	Temperature ° C.		Attacked Kernels (Per Cent)
	Noninfested	Infested	
4	17.7	26.7	34.6
5	16.1	27.0	55.4
6	15.7	28.2	85.8
7	17.0	30.8	97.4
8	20.9	30.5	99.2
9	20.5	29.8	100

the seventh month when the inspection showed 97.4% of the grain attacked. Thereafter the temperature differential decreased. Heating of grain is undoubtedly an important factor in rate of population increase.

SUMMARY.—Studies on the biology and habits of *Pagioceris fiorii* (Egger) in stored soft corn were made in Quito, Ecuador, during 1954 and 1955. This is a small beetle about 3 mm. long of the family Scolytidae. Both the adults and the larvae feed on the corn. The average minimum period from adult to adult averaged 46.4 days. The period from the newly emerged adult until all F_1 adults emerged averaged 70.8 days. The length of life of adults averaged 60.2 days. The average length of the life cycle was 122.5 days.

The total production from one female averaged 75 adults in the F_1 generation. It was calculated that on the average one adult female produces 1200 adults in 4 months, 2250 adults in 5 months, 35,000 adults in 6 months and 78,000 adults in 7 months. An average of 6.2 kernels of shelled corn was attacked by each adult. The temperature of stored corn with about one-third of the kernels attacked was 9° C. higher than the non-infested corn.

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Insecticide Control of Green June Beetle Larvae Attacking Ladino Clover Pastures: Residues on Foliage and Accumulation in Swine Tissue[1]

T. B. DAVICH,[2] A. S. TOMBES[3] and R. H. CARTER[4]

The green June beetle, *Cotinis nitida* (L.), is an important pest of Ladino clover in Virginia and in several southeastern states. Damage consists of loosening of the roots and uprooting as a result of extensive larval burrowing in the upper 4 inches of the soil. The organic matter content of the soil may be greatly reduced by heavy infestations.

Howe & Campbell (1953a) published an account of larval damage to Ladino clover pastures and results of tests to control the insect. They found that aldrin, dieldrin or heptachlor at 2 pounds per acre, lindane at 0.67 pound, or toxaphene at 20 pounds, provided excellent control when applied to the soil surface shortly before the eggs hatched in early July. Effective control was obtained with insecticides in mixture with fertilizer, as granules, as dusts and as sprays. These insecticides were ineffective at the same dosages if applied later in the season against the hardier third instar larvae. The authors reported, "Limited residue analyses of forage to which four formulations containing aldrin had been applied showed an average of 0.7 p.p.m. remained after 3 weeks exposure." No further details were presented concerning residues.

Dominick (1952) reported only fair control of third instar larvae in tobacco plant beds with 1.9 pounds per acre of aldrin, dieldrin or heptachlor. Jewett (1951) reported ineffective control of the hardy third instar larvae with 5 pounds of aldrin per acre to hand-infested pasture plots. Howe & Campbell (1953b) state that in insectary tests first instars were approximately 10 times as susceptible to aldrin as third instars and 5 times as susceptible as second instars.

In 1953–55 various insecticides were investigated to determine the minimal effective dosages for control of first and second instar larvae of the green June beetle in Ladino clover-orchard grass pastures in southeastern Virginia. Data were obtained on aldrin dissipation from Ladino clover following application of dusts and granules, and on accumulation of this chemical in swine, following grazing on Ladino clover and Ladino clover-orchard grass pastures treated with granules.

TESTS IN 1954 AGAINST FIRST AND SECOND INSTAR LARVAE.—Aldrin, dieldrin and heptachlor were each tested against first instar larvae at dosages of 1.0, 1.5 and 2.0 pounds per acre. These toxicants were formulated on 15/30 and 30/40 mesh attapulgite, and 15/30 and 30/60 mesh tobacco. The attapulgite formulations reported in this paper were RVM-AA grade. All formulations were at 2% strength. Application was made with a tractor-pulled fertilizer distributor on July 28. Within 2 days of application a cursory examination revealed large numbers of dead and moribund larvae in almost all of the treated plots. The reduction in population was estimated to be in excess of 90%, even at the lowest dosage, for all three toxicants. Many of the dead and moribund larvae were second instars, a result that was not expected at the 1.0-pound dosage on the basis of previously published reports.

Larval development was closely followed and a small-plot test was conducted against second instars. Three fairly distinct larval sizes and three definite head-width sizes were found on August 6. The head capsule measurements were 1.3, 2.0 and 3.4 mm., indicating the presence of all three larval instars. At the time 94.5% of the larvae were second instars while the remainder was evenly divided between the first and third. The insecticides were

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[2] Associate entomologist, Virginia Agricultural Experiment Station. Now with Entomology Research Branch, Agr. Res. Serv., U.S.D.A.

[3] Research assistant, Virginia Agricultural Experiment Station and the Entomology Research Branch, Agr. Res. Serv., U.S.D.A.

[4] Chemist, Entomology Research Branch, Agr. Res. Ser., U.S.D.A.

applied on August 6, with a rotary hand duster. There were four replications in a randomized complete block design. Each plot was 9 by 25 feet. The foliage ranged up to 10 inches in height and included weeds which were seriously competing with the pasture. Extensive larval burrowing and pasture damage were evident. Population estimates were obtained on September 17, or 6 weeks after treatment, by excavating a randomly selected area, 1 by 2 feet by 6 inches deep, in each plot and counting the the larvae. The results of this test are presented in table 1.

TESTS IN 1955 AGAINST FIRST AND SECOND INSTAR LARVAE.—The excellent control of second instar larvae obtained during the 1954 season from low dosages of the various insecticides tested prompted further dosage studies in 1955. The 1955 test conducted against first instar larvae contained five replications in a balanced lattice square design. Each plot was 12.5 by 100 feet. The foliage ranged from 6 to 10 inches in height, and included weeds along with the Ladino clover and orchard grass. The applications were made on July 18 with an eight-nozzle, tractor-mounted duster with the nozzles set high to produce a broadcast pattern. Population estimates were obtained on August 31 by excavation as described previously. Results of this test are presented in table 1.

The test against second instars was conducted on a Ladino clover-orchard grass pasture which was clipped low prior to treatment. There were four replications in a randomized complete block design. Each plot was 12 by 30 feet. The applications were made on August 8 with a rotary hand duster, and larval counts were obtained on September 14 by excavation. Table 1 shows the results of this test.

Table 1.—Control of green June beetle larvae in Ladino clover-orchard grass pastures following application of granulated insecticides. Holland, Virginia.

INSECTICIDE	TOXICANT LB./A.	FORMULATION	LARVAE PER SQUARE FOOT	PER CENT CONTROL
1954 test against second instar larvae				
Aldrin	1.0	5% on 30/60 attapulgite	0	100.0
Chlordane	2.7	5% on 30/40 attapulgite	0	100.0
Dieldrin	1.0	5% on 30/60 attapulgite	0	100.0
Dieldrin	1.0	5% on 30/60 tobacco	0	100.0
Endrin	0.5	2% on 30/60 tobacco	0	100.0
Heptachlor	1.0	5% on 30/60 tobacco	0	100.0
Isodrin	0.5	2% on 30/60 tobacco	0	100.0
Toxaphene	2.9	10% on 30/40 attapulgite	0	100.0
DDT	3.4	5% on 30/40 attapulgite	0.1	98.0
Heptachlor	1.2	10% on 16/60 vermiculate[a]	0.4	91.8
Heptachlor	1.1	5% on 30/60 attapulgite	0.8	83.7
Aldrin	1.0	5% on 30/60 tobacco[a,b]	1.0	79.6
Malathion	2.9	10% on 30/40 attapulgite	2.6	46.9
Untreated	—	—	4.9	—
1955 test against first instar larvae				
DDT	2.0	10% on 30/40 attapulgite	0	100.0
Dieldrin	0.5	2% on 30/60 vermiculite	0	100.0
Endrin	0.25	1% on 30/60 vermiculite	0	100.0
Isodrin	0.2	1% on 30/60 vermiculite	0	100.0
Endrin	0.4	2.5% on 30/60 vermiculite	0.1	97.2
Isodrin	0.4	2.5% on 30/60 vermiculite	0.1	97.2
Toxaphene	2.0	10% on 30/60 vermiculite	0.1	97.2
Toxaphene	3.8	10% on 30/60 vermiculite	0.2	94.4
DDT	2.5	10% on 30/40 attapulgite	0.3	91.7
Isodrin	0.9	2.5% on 30/60 vermiculite[a]	0.3	91.7
Toxaphene	1.0	5% on 30/60 vermiculite	0.3	91.7
Aldrin	0.5	2% on 30/60 vermiculite[a]	0.4	88.9
DDT	1.0	10% on 30/40 attapulgite	0.8	77.8
Heptachlor	0.5	2% on 30/60 vermiculite	0.8	77.8
Endrin	0.7	2.5% on 30/60 vermiculite[a]	1.0	72.2
Untreated	—	—	3.6	—
1955 test against second instar larvae				
Dieldrin	0.4	2.5% on 30/40 attapulgite	0	100.0
Dieldrin	0.6	2.5% on 30/40 attapulgite	0.1	96.9
Heptachlor	0.4	2% on 30/60 vermiculite	0.2	93.8
Aldrin	0.8	2.5% on 30/40 attapulgite[a]	0.4	90.6
Dieldrin	0.2	1% on 30/40 attapulgite	0.6	84.4
Toxaphene	1.5	5% on 30/60 vermiculite	0.8	81.2
Aldrin	0.5	2.5% on 30/40 attapulgite	1.0	75.0
Heptachlor	0.8	2% on 30/60 vermiculite[a,b]	2.2	43.8
Aldrin	0.2	1% on 30/40 attapulgite	2.5	37.5
Toxaphene	0.8	5% on 30/60 vermiculite	2.6	34.4
Heptachlor	0.25	1% on 30/60 vermiculite	3.2	18.8
Toxaphene	2.0	5% on 30/60 vermiculite	3.4	15.6
Untreated	—	—	4.0	—

[a] Only one replicate in these treatments contained larvae; this is probably attributable to migration from heavily infested, untreated, border areas of pasture.

[b] Two sides of one replicate were exposed to migration from border areas in these treatments.

INSECTICIDE RESIDUE TESTS.—*Aldrin residues on Ladino clover.*—Dissipation of aldrin from Ladino clover treated with dusts and granulated formulations was studied during the 1955 season. An excellent stand of Ladino clover, 3 to 4 inches tall, was treated on the morning of August 1, following a heavy dew. A rotary hand duster was utilized in making the applications. The experiment contained four replications in a randomized complete block design, and individual plots were 15 by 40 feet. Table 2 shows the treatments in this residue experiment. Forage samples were taken immediately after application and 3, 7, 14 and 21 days following application. The sampled area, which varied from 9 to 20 square feet, was clipped of vegetation to within 1 inch of the soil surface. The samples were weighed in the field immediately after clipping, placed in polyethylene bags and stored in a freezer (approximately −15° C.) until ready for extraction. Two replicates of the experiment were analyzed in a laboratory of the Virginia Agricultural Experiment Station, and the other two at the Residue Analysis Laboratory, Shell Chemical Corporation. Residues were estimated by the phenyl azide-photometric method at both laboratories. Table 2 presents the results of this experiment.

Insecticide residues in swine, 1953 test.—Data on the accumulation of aldrin in swine, following grazing on pastures treated with granulated formulations, were obtained during 1953. Swine were selected as experimental animals because of their importance to the farm economy of southeastern Virginia, the high proportion of the swine population grazed on Ladino clover or Ladino clover-orchard grass pastures, and the possibility of their accumulating insecticides from soil treatment due to their rooting habits. Four Ladino clover pastures, each 50 by 150 feet, were fenced off and provided with water facilities and self-feeders. Foliage growth was estimated at 2 to 3 inches tall. Three of the pastures were treated with aldrin granules while the fourth was left untreated. Applications were made on July 18 and 20 with a rotary hand duster. Formulations and application data are presented in table 3. Eight young swine, all litter mates, were classed into two weight groups and one animal from each group was randomly assigned to each pasture on July 22. The swine in the heavier weight group averaged 64 pounds, while those in the lighter weight group averaged 51 pounds. The test animals were removed from pasture on October 6, slaughtered, and various tissues removed for quick freezing prior to analysis for insecticide content.

The mean daily weight gain and feed consumed were, within the limits of experimental error, unaffected by treatment. Although appearing perfectly normal during the grazing period, a difference in rooting habits was

Table 2.—Aldrin residues[a] on Ladino clover samples taken periodically after pasture treatment. Holland, Virginia, 1955.

Aldrin Per Cent	Aldrin Lb./A.	Interval Between Treatment and Sampling (Days)									
		0		3		7[b]		14[b]		21	
Dust Formulations[c]											
2.5	0.5	1.2	1.6	0.41	0.54	0.15	0.18	<.10	<.10		
5.0	.9	2.1	3.6	.81	.89	.36	.52	<.10	<.10		
5.0	2.1	3.3	6.2	1.5	2.7	.53	1.5	.15	.19	<.10	<.10
Attapulgite formulations[c]											
2.5	.5	.71	.50	.23	.40	.17	.24	<.10	.14		
5.0	.8	.97	.92	.21	.46	.23	.46	<.10	.15		
5.0	2.2	2.3	2.0	1.4	1.6	1.0	.95	.26	.44	<.10	
Vermiculite formulations[c]											
2.0	.5	.60	.89	.41	.52	.11	.19	<.10	<.10		
5.0	1.0	1.1	1.7	.67	1.3	.40	.48	<.10	.16		
5.0	2.4	1.4	2.9	.54	1.2	.51	.80	.15	.68	<.10	

[a] Expressed as p.p.m. on green weight basis; each value is the average of two replicates. Virginia Agricultural Experiment Station results in left column under each interval and Shell Chemical Corporation results in right column.
[b] Rainfall total 6.6 inches between 7 and 14 day interval and 2.8 inches following 14 day interval.
[c] Dusts formulated on 325 mesh attapulgite; attapulgite on 30/40 mesh granules and vermiculite on 30/60 mesh granules.

noted. The swine grazed on the untreated pasture and on the one which received 1.9 pounds of aldrin per acre very nearly destroyed the clover stand by extensive rooting. The pastures which received the two higher dosages showed almost no signs of rooting. At the time of slaughter no gross pathological differences between swine grazed on treated and untreated pastures were observed in the liver, stomach or kidneys. Tissue from the heavier weight group. which averaged 206 pounds at slaughter, was analyzed at the Residue Analysis Laboratory, Shell Chemical Corporation; while tissues from the lighter weight group, which averaged 176 pounds at slaughter, were analyzed by the Entomology Research Branch, U.S. Department of Agriculture. The tissues analyzed and the results of the analyses are shown in table 3.

Insecticide residues in swine, 1954 test.—The 1954 swine residue experiment utilized four toxicants formulated on granulated carriers. The data are presented in table 4. Individual Ladino clover-orchard grass pastures were 60 by 120 feet and provided with water facilities and self-feeders. Insecticide applications were made on July 31 and August 2, with a rotary hand duster. Young swine, averaging approximately 40 pounds, were added 1 or 2 days following treatment; while a second group, averaging 60 pounds, was added 14 or 16 days following treatment. All animals were removed from pasture on December 13, at which time the first group had grazed an average of 132 days and the second group 119 days. On December 14 and 15 the animals were slaughtered and samples of fat back were placed in polyethylene bags and frozen prior to analysis. No gross differences were observed in the internal organs between swine grazed on treated and untreated pastures. Food consumption and weight gain were normal for all swine under test. Results of the analyses are shown in table 4.

Discussion of Results.—*Chemical control tests.*—As shown in table 1, most of the chlorinated hydrocarbon insecticides tested gave effective control of first and second instar larvae. There are a number of apparent inconsistencies in the data presented, which can perhaps be explained. In the 1954 test 1 pound of aldrin on tobacco granules appeared to give control inferior to the same

Table 3.—Aldrin and dieldrin accumulation in fat and livers of swine grazed 76 days on Ladino clover pastures treated with granulated aldrin. Holland, Virginia, 1953.

Aldrin Lb./A.	Formulation	Days from Pasture Treatment to Exposure	Shell Chemical Corporation				Entomology Research Branch	
			Tissue Analyzed	Aldrin[a] p.p.m.	Dieldrin[b] p.p.m.		Tissue Analyzed	Aldrin[c] p.p.m.
1.9	2% on 30/60 attapulgite	2	Fat back	<.1	0.2	0.4	Fat back	2.2
			Liver	<.1			Omental fat	1.2
7.0	5% on 30/60 tobacco	4	Fat back	<.1	0.62	0.9	Fat back	Lost
			Liver	<.1			Omental fat	1.0
14.8	5% on 30/60 attapulgite	2	Fat back	<.1	1.82	2.7	Fat back	2.0
			Liver	<.1			Omental fat	5.1
Untreated			Fat back	<.1	<.2	<.2		
			Liver	<.1				

[a] Analyzed by phenyl azide-photometric method.
[b] Left column results by reduction-phenyl azide-method, right column by bioassay method using house flies.
[c] Analysis based on organic chlorine.

Table 4.—Insecticide accumulation in subcutaneous fat of swine following grazing on Ladino clover-orchard grass pastures treated with granulated insecticides. Holland, Virginia, 1954.

Insecticide	Toxicant (Lb./A.)	Formulation	Days from Treatment to Exposure	Grazing Period (Days)	Toxicant Found, P.P.M.	
					Shell Chemical Corporation[a]	Entomology Research Branch[b]
Aldrin	2.0	5% on 30/60 attapulgite	1	132	<.1	0
	4.0	—Do—	1	132	<.1	0.4
	4.0	—Do—	14	119	<.1	0.5
Dieldrin	1.7	—Do—	2	133	0.48	3.0
	1.7	—Do—	16	119	0.22	0.1
	3.9	—Do—	14	119	0.25	1.1
Heptachlor	3.6	5% on 30/60 tobacco	1	132		0.9
Toxaphene	16.0	20% on 30/40 attapulgite	2	133		1.0

[a] Phenyl azide-photometric method for aldrin and reduction-phenyl-azide method for dieldrin.
[b] Analysis based on organic chlorine.

toxicant dosage on attapulgite. In 1955 toxaphene and heptachlor against second instars and endrin against first instars appeared to provide ineffective control at high dosages and effective control at low dosages. Larval migration from heavily infested, untreated, border areas appeared to be responsible for the anomalies observed. If the organic matter in an area becomes depleted, larvae will leave their burrows and travel some distance in search of better feeding areas. Those larvae entering treated plots shortly before excavation undoubtedly were exposed only to sublethal levels of toxicants, due to toxicant dissipation or greater resistance of the third instars. Only third instar larvae were found at the time of excavation.

Aldrin residues on Ladino clover.—Aldrin residues (table 2) were higher, in general, on the samples analyzed by the Shell Chemical Corporation than on those analyzed by the Virginia Agricultural Experiment Station. Part of this discrepancy may be attributable to application and sampling variation, since individual rather than composite samples were analyzed. Recovery of aldrin following addition of known amounts to clover extract was higher by Shell Chemical Corporation, as shown in table 5. Undoubtedly this accounts for much of the variation in amounts of residues found between the two laboratories. When subjected to statistical analysis the average residues obtained by Shell Chemical Corporation were highly significantly greater ($P = <.01$) than those found by the Virginia Agricultural Experiment Station for the 0- and 3-day intervals and significantly greater ($P = <.05$) for the 14-day interval. There was no significant difference between replicates analyzed by the Virginia Agricultural Experiment Station, whereas significant differences occurred between replicates in the Shell Chemical Corporation analyses. On the 7-day-interval values both laboratories were essentially in agreement. Sensitivity of the analytical method, at both laboratories, was 0.1 p.p.m. Residues less than this level, for discussion purposes, are considered to be zero. The apparent aldrin found in untreated check samples (table 5) was negligible.

Table 5.—Recovery of aldrin from Ladino clover extract following addition of known amounts, and apparent aldrin found in untreated check samples.

Laboratory	P.P.M. Aldrin Added to Extract	Per Cent Recovery from		Apparent Aldrin P.P.M.
		Granules	Dust	
Shell Chemical Corporation	0.1	91	80	
	.3	115	93	
	0	(Untreated)		0.02
Virginia Agricultural Experiment Station	.1	85		
	.3	83		
	0	(Untreated)		0.03

Samples taken on the day of application and 3 days later showed significantly higher aldrin residues for the 2.1-pound dosage, applied as a dust, than for the remaining eight treatments. On the day of application the 0.9-pound dosage applied as a dust, resulted in significantly higher residues than the 0.8-pound on attapulgite granules or the 1.0-pound dosage on vermiculite granules. Although other differences in the magnitude of residues between formulations, dosages and sampling dates might be indicated, it is felt that the 14- and 21-day intervals are the most important for the purpose of recommending control measures. Both laboratories reported zero residues on identical sampling dates for the three dust treatments and for the lowest dosage on vermiculite. Residues found on the remaining treatments were at the zero level 14 or 21 days after application, for the Virginia Agricultural Experiment Station series. No additional samples, beyond the 14-day interval, were shipped to the Shell Chemical Corporation for analysis. On the basis of the dissipation observed in the Shell Chemical Corporation series, however, it appears reasonable to assume that only the highest aldrin dosage on vermiculite and attapulgite could result in measurable residues 21 days following application.

Insecticide residues in swine.—The specific analytical method for aldrin, sensitive to 0.1 p.p.m., failed to detect residues in tissues of swine which had grazed for extended period on pastures treated with dosages up to 14.8 pounds per acre (table 3). Varying amounts of aldrin were found when analysis was based on organic chlorine. This method was sensitive to approximately 0.5 p.p.m. Bann *et al.* (1955) have shown that aldrin is converted to dieldrin in the animal body. For this reason analysis for dieldrin also was performed on swine tissue obtained from animals which had grazed on aldrin-treated pastures. The bioassay procedure resulted in dieldrin residues higher than those found by chemical analysis. Both analytical

methods were sensitive to 0.2 p.p.m. of dieldrin. As the aldrin dosage applied to pastures was increased, increasing amounts of dieldrin were found in the tissue (table 3). No residues of dieldrin were found, however, in the fat of swine grazed on aldrin-treated pastures in the 1954 test (table 4). Swine grazed on aldrin-, dieldrin-, heptachlor- and toxaphene-treated pastures accumulated residues of these toxicants in subcutaneous fat (table 4).

SUMMARY.—A number of granulated insecticide formulations were tested against first and second instar green June beetle larvae attacking Ladino clover-orchard grass pastures in southeastern Virginia. Three factors precluded positive establishment of minimal dosages for control of first and second instar larvae: (1) larval migration into treated plots following dissipation of toxicant, (2) the inherently greater resistance to insecticides exhibited by third instar larvae, and (3) sampling variation. However, dieldrin at dosages as low as 0.4 pound per acre, and aldrin or heptachlor at 1.0 pound, usually resulted in effective control of second instar larvae. These should be extremely effective against first instars if applied shortly after egg-hatching. One hundred per cent control of second instar larvae was attained with endrin or isodrin at 0.5 pound per acre, and of first instar larvae with 0.25 pound of endrin or 0.2 pound of isodrin. Chlordane at 2.7 pounds per acre provided 100% control of second instar larvae. Toxaphene, although somewhat erratic in control of second instars, provided effective first instar control when applied at 2.0 pounds per acre. DDT provided effective first and second instar control at 2.0 and 3.4 pounds per acre, respectively. Malathion at 2.9 pounds per acre was ineffective against second instar larvae.

Aldrin resulted in higher initial foliage residues when applied in dust form than in granulated attapulgite or vermiculite formulations. However, all three formulations, at dosages up to 1.0 pound per acre, either resulted in residues less than 0.1 p.p,m. 14 days following application, or could be expected to result in such residues within 21 days following application. All three formulations, at dosages of 2.1 to 2.4 pounds per acre, required in excess of 14 days to dissipate to levels below 0.1 p.p.m.

Various tissues were obtained from swine which had grazed for extended periods on Ladino clover or Ladino clover-orchard grass pastures treated with granulated insecticide formulations. Aldrin dosages up to 14.8 pounds per acre failed to result in accumulation of measurable residues when analysis was based on the specific procedure. The analysis based on organic chlorine, and the specific analysis for dieldrin, showed toxicants had accumulated in the fat obtained from swine grazed on aldrin-treated pastures. Increasing amounts of toxicant were found as the aldrin dosage was increased. Fat tissue from swine which had grazed on pastures treated with 1.7 and 3.9 pounds of dieldrin per acre accumulated measurable residues when analyzed either by the specific method or by the organic chlorine method. Heptachlor, applied at 3.6 pounds per acre, and toxaphene, applied at 16.0 pounds per acre, accumulated in swine fat at levels of 0.9 and 1.0 p.p.m., respectively.

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BOOK REVIEW

CHEMISTRY AND USES OF PESTICIDES. By E. R. deONG. 2nd Edition. vii+334 pages. Reinhold Publishing Co., New York. 1956. $8.75.

The first edition of this book was entitled "Chemistry and Uses of Insecticides." The author has included fungicides, herbicides, rodenticides, repellents and seed disinfectants in the present edition. To make room for this material the chapters on "Arsenical Compounds," "Copper Compounds," "Sulfur and Its Compounds," "Miscellaneous Inorganic Compounds" and "Petroleum and Its Products" have been rewritten and condensed. A new chapter "Organic Herbicides and Rodenticides" has been added and the other pesticides are included in the chapter "Synthetic Organic Insecticides and Fungicides; Repellents; Seed Protectants." Almost half of the book is devoted to these two chapters. The chapters, "Fumigants and Fumigation," "Plant Derivatives," and "Cold, Heat, Dehydration and Radiation as Pesticides have been rewritten and expanded.

The style of the book remains the same as in the first edition. The pesticidal chemical is given and this is followed by its physical properties, formulations and uses. The ample index is used to direct the reader to the principal chemicals used in the control of any given pest. This system is not followed in the case of repellents, to which subject only two and a half pages are devoted. The allied subject, attractants, is neglected entirely.

The Appendix contains a useful "Dictionary of Pesticides," a summary of the Food and Drug tolerances of pesticide residues on fresh fruits and vegetables, and antidotes to be used in case of accidental poisoning with pesticides.

This handbook should be very useful to anyone concerned with production or use of pesticides. The information on each individual pesticide is well organized and easy to locate. Each chapter is backed up with well chosen references which direct the reader to more detailed information.

ELMER E. FLECK

SCIENTIFIC NOTES

Field Tests of Insecticidal Sprays for Control of Cabbage Looper on Lettuce[1]

F. F. Bibby,[2] *Arizona Fertilizers, Inc., Phoenix, Arizona*

Field tests were begun at Queen Creek, Arizona, in 1956 primarily to determine whether a perennial complaint of lack of control of cabbage looper (*Trichoplusia ni* (Hbn.)) with conventional insecticides at currently recommended dosages in the Salt River Valley of Arizona was caused by ineffectiveness of the insecticides themselves, or by much-blamed faulty applications of them. The tests, results of which are reported herein, developed into more of a procedure for screening, substitution, and changing of dosages instead of following the original objective; though extreme care was always taken to have the insecticides applied well.

Materials and Methods.—At the beginning of the tests there were 8 plots of 3 acres each which consisted of 32 beds (double-rows) of lettuce (with two exceptions: one plot, slightly smaller, and the other, larger by one-half). Of the 8 plots there were 2 replicates of toxaphene at 2 pounds per acre; 3 of toxaphene-DDT at 2 pounds and 1 pound, respectively, and 3 of DDT at 1 pound, arranged in the order as given in table 1. The first application was made September 19 to 20 with a tractor-mounted sprayer fitted with two hollow-cone nozzles per row and which treated four beds (8 rows) per trip. The rig was calibrated to spray 10 gallons per acre at a pressure of 60 p.s.i. when traveling at 5 m.p.h. The application was made soon after the lettuce had been thinned and when it was free of lepidopterous larvae, evidently as a result of a recent commercial application of toxaphene-DDT dust. However, the crop bore an abundance of eggs, mostly of cabbage looper, and some of beet armyworm (*Laphygma exigua* (Hbn.)) and of granulate cutworm (*Feltia subterranea* (F.)).

The amount of toxicant applied was determined by adding the correct amount of emulsifiable concentrate of each insecticide to the number of gallons of water applied per acre. Infestation in each plot was determined by carefully examining 30 consecutive plants at each of two points in the center rows. Each plant bearing one or more live cabbage looper or beet armyworm larvae, regardless of size, was recorded as infested.

Results.—With a few exceptions the first treatments did not give ample control. In an attempt to obtain better control with the same insecticides or combinations of them each of the original plots was divided equally (with one exception) and the rate of the toxicant doubled. The one exception was in Plot 1, to which a combination of malathion-Perthane at 1 pound of each to the acre was applied. This application and all others that followed were made like the first, except that the number of nozzles per row and the number of gallons of spray per acre were doubled, making four nozzles per row and 20 gallons of spray per acre.

After doubling the dosage of the toxicants and substituting malathion-Perthane for toxaphene in a portion of one plot considerable improvement in control was indicated in all plots except where toxaphene was applied at 4 pounds per acre. The malathion-Perthane combination (1 pound of each to the acre) gave outstanding control as shown in table 1.

For the purpose of further screening, and in order to save the commercial lettuce field from further depredation, additional changes in the formulations applied were made after the second application. Because neither toxaphene (at either rate) nor DDT at the lower dosage gave satisfactory control, other insecticides or combinations were substituted for them as follows: the malathion-Perthane combination was applied to the remaining portion (except 14 short rows left untreated as a check for 2 days) of Plot 1; malathion at 1 pound per acre to one-half of

Table 1.—Sequence and results of field tests of insecticidal sprays to lettuce for control of cabbage looper at Queen Creek, Arizona, 1956.

Plot No.	Insecticide	Dosage Lbs./A.	Date Applied	Per Cent of Plants Infested	Date of Count
1. a, b, & c	Toxaphene	2	Sept. 19	15.0	Sept. 24
				30.0	Sept. 26
a & c		2	Sept. 28	25.0	Oct. 1
				96.7	Oct. 4
b	Malathion-Perthane	1–1		8.3	
				16.7	
a		1–1	Oct. 5	23.3	Oct. 7
c	Not Treated			98.3	
	Malathion-Perthane	1–1	Oct. 7	28.3	Oct. 12
a		1–1	Oct. 13	10.0	Oct. 16
b		1–1		1.7	
c		1–1		8.3	
a & b	Toxaphene-DDT	2–1	Sept. 19	5.0	Sept. 23
				13.3	Sept. 26
a		2–1	Sept. 28	5.0	Oct. 1
b		4–2		0.0	
a		2–1		30.0	Oct. 4
b		4–2		16.7	
a		2–1	Oct. 7	40.0	Oct. 9
b		4–2		5.0	
a		2–1		31.7	Oct. 12
b		4–2		6.7	
3. a & b	DDT	1	Sept. 20	0.0	Sept. 23
				16.7	Sept. 26
a		1	Sept. 28	18.3	Oct. 1
b		2		1.7	
a		1		61.7	Oct. 4
b		2		5.0	
a	Perthane	1	Oct. 7	83.3	Oct. 9
b	DDT	2		28.3	
a	Perthane	1		93.3	Oct. 13
b	DDT	2		25.0	
4. a & b	Toxaphene	2	Sept. 19	3.3	Sept. 24
				38.7	Sept. 26
a		2	Sept. 28	25.0	Oct. 1
b		4		21.7	
a	Malathion	1	Oct. 7	71.7	Oct. 10
b	Perthane	1		90.0	
a	Malathion	1		73.3	Oct. 14
b	Perthane	1		76.7	
5. a & b	Toxaphene-DDT	2–1	Sept. 19	0.0	Sept. 23
				6.7	Sept. 26
a		2–1	Sept. 28	0.0	Oct. 2
b		4–2		1.7	
a		2–1		25.0	Oct. 4
b		4–2		10.0	
a		2–1	Oct. 7	26.7	Oct. 10
b		4–2		1.7	
a		2–1		13.3	Oct. 14
b		4–2		5.0	
6. a & b	DDT	1	Sept. 20	1.7	Sept. 23
				35.0	Sept. 28
a		1	Sept. 30		Rained off
b		2	Sept. 30		Rained off
a		1	Oct. 1	70.0	Oct. 5
b		2		20.0	
a	Malathion	2	Oct. 9	48.3	Oct. 11
b	DDT	2	Oct. 9	1.7	
7. a & b	Toxaphene-DDT	2–1	Sept. 19	0.0	Sept. 23
				11.7	Sept. 28
a		2–1	Oct. 1	18.3	Oct. 5
b		4–2		11.7	
a		2–1	Oct. 9	10.0	Oct. 11
b		4–2		0.0	
8. a & b		1	Sept. 20	0.0	Sept. 24
a		1	Sept. 30		Rained off
b		2			Rained off
a		1	Oct. 1	63.3	Oct. 5
b		2		10.0	
a	Perthane	1.75	Oct. 9	65.0	Oct. 11
b	DDT	2		3.3	

Paid Paper.

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[2] The writer is grateful to Kenneth Tipling and Stuart Evans for rendering invaluable service in renovating and calibrating the sprayer and for other assistance in making the tests, and to E. E. Hawes, the grower, for his most hearty cooperation in every respect.

Plot 4 and Perthane at 1 pound per acre to the other half; malathion at 2 pounds per acre to one-half of Plot 6; and Perthane at 1.75 pounds per acre on a similar portion of Plot 8.

The portion of Plot 1, designated as "c" in the table, after having received two applications of toxaphene at 2 pounds per acre had 96.7% of the lettuce plants infested with one or more larvae per plant on October 4. It was left untreated October 5 and within 2 days developed an increase in infestation to 98.3%. It was treated with the malathion-Perthane combination (1 pound of each per acre) on October 7, and by October 12 the infestation had decreased to 28.3%. To this portion of Plot 1 and to the remaining portions (a & b), the final application of malathion-Perthane was made on October 13 with a further reduction in infestation ranging from 1.7 to 10% as shown in table 1.

The apparent differences in infestation of plots treated alike at the beginning of the tests were probably caused by differences in growth of the lettuce resulting from the process of thinning which extended over a period of a week, progressing from Plot 1 to Plot 8. Irrigation followed in the same order and extended over a similar period. These conditions probably left the first plots more attractive for oviposition and with less effective residues remaining from the commercial application of insecticide made September 16, three days before the tests discussed herein were started. As time passed plots from one side of the field to the other became more uniformly infested. There was also an unavoidable difference in the interval between applications to various plots because of the progress of the irrigation from one side of the field to the other that may have affected the results. Regardless of these factors the results indicate that toxaphene did not give satisfactory control at a dosage of either 2 or 4 pounds per acre; that the toxaphene-DDT combination of 4 pounds and 2 pounds, respectively, per acre was more effective than the toxaphene-DDT combination at 2 pounds and 1 pound, respectively. The lower dosage of the toxaphene-DDT combination was more effective than either toxaphene or DDT applied separately at 2 pounds and 1 pound, respectively, per acre, and even more effective than toxaphene alone at 4 pounds per acre, but not better than DDT alone at 2 pounds per acre. DDT applied at 1 pound per acre was unsatisfactory but at 2 pounds it served well against young larvae, but was less effective than the stronger combination of toxaphene-DDT and somewhat more effective than the weaker combination of these materials.

The malathion-Perthane combination at the rate of 1 pound of each per acre was outstanding. It killed the larger larvae, and gave a higher percentage of control than any other insecticide or combination of insecticides tested. Neither malathion at 1 or 2 pounds per acre nor Perthane at 1 or 1.75 pounds per acre gave satisfactory control.

Occurrence of the Boll Weevil in the Big Bend of Texas[1]

O. T. ROBERTSON,[2] *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*[3]

The boll weevil, *Anthonomus grandis* Boh., was seldom found prior to 1953 in the Big Bend area of Texas. The nearest infestation where it caused damage was at Delicias, Chih., Mexico, about 100 miles away. It is seldom a pest in the High Plains or west of the Pecos River in Texas, and has never caused commercial damage west of the Big Bend in the United States. An outbreak observed in 1953–55 is of significance since it occurred more than 200 miles west of cotton-producing areas in Texas where damage usually occurs.

The cotton-growing land of the Big Bend is the irrigated acreage along the Rio Grande River in Brewster and Presidio Counties. This cultivated land, beginning at Castolon in Brewster County, extends westward about 120 miles to Candelaria in Presidio County. The western boundary is about 150 miles below El Paso on the Rio Grande. At Presidio, which is located about midway between Castolon and Candelaria, the elevation is 2,594 feet, and the average annual rainfall is 10.23 inches. Winter temperatures frequently drop to around 20° F. with a record low of 11° F. Summer temperatures commonly rise above 100° with a record high of 116°. Cotton has been grown in the area since 1915.

During the period of pink bollworm investigations by Federal and State authorities in the Big Bend, from 1927 to 1947, occurrence of the boll weevil was so rare that it was not mentioned in the station reports. W. L. Owen, of the Texas Agricultural Experiment Station, reported a specimen collected at Presidio in June 1935 and J. H. Russell, of the Bureau of Entomology and Plant Quarantine, collected specimens from gin trash in the fall of the same year. The author has observed many cotton fields in the area at frequent intervals during every growing season since 1936 and did not see any sign of the boll weevil prior to 1953.

In early November of 1953 a boll weevil infestation was found at Redford, about 20 miles southeast of Presidio. Approximately 800 acres of cotton was grown in the Redford section extending for a distance of 7 miles along the Rio Grande. The infestation was heaviest in the southeastern part and became lighter toward the northwest. The infestation on about 20 acres of cotton was sufficiently high to cause damage to late bolls which no doubt resulted in some loss in yield.

In 1954 periodic surveys were made to determine the seasonal abundance of the boll weevil in the Redford section. No sign of boll weevils was found in June. In July one square was found which resembled an egg puncture but no larva was found in the square. Observations on August 11 continued to show a very low infestation in this section. On September 15 square-puncture counts were made in 18 fields in the Redford and Presidio sections. In the Redford section the percent of squares punctured ranged from 70 to 96, averaging 84.5, and insecticides were being applied. These applications were the first ever made for the control of the boll weevil in the Big Bend. In the Presidio section square infestation in 12 fields ranged from 0 to 82% averaging 11.2, but no insecticides were used. The highest infestation in this section was in the southeastern part which is nearer the Redford section. No square punctures or adult weevils were found northwest of Presidio. Early in November a light infestation was found 20 miles northwest of Presidio.

In 1955 insecticides were used extensively for control of the boll weevil in the Redford and Presidio sections, as many as 20 applications being made in some fields. At Presidio the first adult boll weevil was found on June 7, and three punctured squares were found on June 23. On July 14 several fields were checked in this section and punctured squares were found in all fields. Five weeks later square-puncture counts were made in 23 fields in both sections. The percent of squares punctured ranged from 0 to 88, averaging 23. The negative records were for fields where five applications of insecticides had been made for control of the pest, and the highest infestations were found in fields where no insecticides had been used. At this time the infestation was still confined within the 1954 infested area. On September 20 and 21 weevils were found in all of 17 fields sampled at points distributed from Castolon to Candelaria. The per cent of squares punctured ranged from 8 to 100, averaging 64. The highest infestation was immediately west of Presidio, where no insecticides had been used, and a 35% infestation was found at Candelaria on the western extremity of the area.

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[2] The assistance of J. H. Russell of the Plant Quarantine Branch, L. D. Harris of the Plant Pest Control Branch, and J. W. Davis and Bobby D. Pell of the Entomology Research Branch (all of the U. S. Department of Agriculture) is gratefully acknowledged.

[3] In cooperation with the Texas Agricultural Experiment Station.

Migration of Larvae of Southwestern Corn Borer on Corn[1]

S. D. HENSLEY[2] and K. D. ARBUTHNOT, *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

Practical insecticidal control of the southwestern corn borer (*Zeadiatraea grandiosella* Dyar) has not been achieved (Walton & Bieberdorf 1948, Walton & Arbuthnot 1954, Rolston 1955). Several insecticides have been found effective, but the large number of applications and amount required make the treatments too expensive to recommend to farmers. As an aid to better timing of insecticide applications, a study of the location and migration of the larvae on corn plants was desirable. The period June and July of 1955 was an excellent time for such a study, because the natural infestation was very low and it was possible to obtain needed populations by manually infesting the plants with eggs. Tests were therefore conducted on the Thomas farm near Stillwater, Okla.

Eggs were deposited on waxed paper by caged moths in a laboratory. These moths had emerged from pupae of field-collected first-generation larvae. Small triangles of the waxed paper, each containing an egg mass, were kept at a constant temperature of 80° F. for 4 days, and observed daily for embryonic development. Fertile eggs are characteristically marked with three red bars that appear approximately 24 hours after deposition; the eggs hatch in about 5 days (Davis *et al.* 1933).

On June 18, 77 masses containing 329 fertile 4-day-old eggs were used to infest plants of the corn hybrid Oklahoma 301 that had been planted on May 3. These plants were 3 feet apart in a single row and averaged 38 inches in height. An egg mass was placed on each plant at one of the following positions: (1) low stalk, approximately 3 inches above the ground; (2) low leaf, the leaf nearest the ground; (3) mid-stalk, approximately 18 inches above the ground; (4) mid-leaf, the leaf nearest the mid-stalk position; (5) high stalk, immediately below the whorl; and (6) high leaf, the uppermost unfurled leaf. The egg masses were attached to stalks at the middle of internodes and to midribs of leaves 3 inches from ligules. Thirteen plants received egg masses in each of the positions 1 to 5, and 12 in position 6.

On June 19, 24 hours later, the hatch was recorded, as shown in table 1. Subsequent observations showed that no more eggs hatched and that about 4% were parasitized by *Trichogramma minutum* Riley, a common egg parasite. In other infestation work 28% of the eggs placed on plants 48 hours prior to hatching were parasitized by *minutum*.

From June 20 to July 6 all the infested plants were dissected to determine where the larvae became established and at what stage of development they tunneled the stalks. Seven plants selected at random were dissected on each of 11 days. Plant height was measured on 5 of these days. The data are presented in table 2.

Regardless of the position where eggs were placed on the plants, first-instar larvae migrated to the plant whorls and remained there until they reached the third instar. During this time they were above the bud, between the moist, compact, immature overlapping leaves of the whorl and in the tissue of the immature tassels. Feeding damage consisted of skeletonization of leaf surfaces and injury to the anthers of the enclosed tassels. Except for one first instar in an ear shoot, all first and second instars were in the plant whorls. Larvae migrated from the whorls and began tunneling into stalks while in the third instar, since five third instars were found in the whorls or partially opened tassels and eight were tunneling into the stalks. All the fourth and fifth instars found were tunneling into or were already inside the stalks.

Apparently southwestern corn borers prefer to tunnel into stalks below the ear zones, as only three tunnels and two larvae were found between the ear zone and tassel. It is believed that early-instar larvae establish themselves in plant whorls because adequate moisture and tender plant tissue for food are most available there. When the leaves have unfurled and the tassels are fully exposed, these moist areas are no longer present, a condition that existed during this study when third-instar larvae migrated. Therefore, it was not possible to determine whether the larvae left the whorls because they became unsuitable as feeding sites or because third instars are inherently disposed to migrate.

Table 1.—Number and per cent hatch of southwestern corn borer eggs at six positions on whorl-stage corn plants.

POSITION	NUMBER OF EGGS PLACED ON PLANTS	PER CENT HATCH
Low stalk	73	86.3
Low leaf	53	84.9
Mid-stalk	47	78.7
Mid-leaf	45	84.4
High stalk	58	75.8
High leaf	53	84.9
Total or Average	329	82.6

Table 2.—Development and location of southwestern corn borer larvae on corn plants dissected at intervals after hatching.

DAYS AFTER HATCH	PLANT HEIGHT (INCHES)	NUMBER OF LARVAE IN INSTAR					LOCATION IN PLANTS
		I	II	III	IV	V	
1	41	14	—	—	—	—	In whorls
2	—	6	—	—	—	—	5 in whorls, 1 in ear shoot (node 5)
3	48	8	—	—	—	—	In whorls
4	—	10	—	—	—	—	In whorls
5	—	9	—	—	—	—	In whorls
6	—	8	3	—	—	—	In whorls
7	55	—	9	1	—	—	In whorls
10	—	—	1	5	—	—	1 second and 2 third instars in whorls, 3 third instars tunneling into stalks
11	60	—	—	6	3	—	1 third instar in tassel, 5 third and 3 fourth instars tunneling into stalks
12	—	—	—	1	9	—	1 third instar in tassel, 9 fourth instars tunneling into stalks
17	66	—	—	—	—	5	In tunnels in stalks

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[2] Also graduate assistant, Oklahoma Agricultural and Mechanical College.

On the Causes of Broken Wings of the House Fly[1]

RICHARD S. PATTERSON, *Department of Entomology, University of Massachusetts, Amherst*

Several investigators, Barber & Starnes (1949) Starr (1953) have noted the occurrence of broken wings among caged house flies, *Musca domestica* L. Since this condition has not been recorded among field flies, it has been supposed that the breakage might be a result of nutritional deficiency, overcrowding, high activity under confined conditions, or a selection of a weak strain of flies. Barber & Starnes suggested that breakage might be a result of the females resistance to attempted mating by the males, since the breakage was confined largely to the latter. They reported that the females with a thrust of the mesothoracic legs during resistance to males were capable of tearing the appendages of the males.

During the process of thrusting the males away the female raises her body from the substrate by extension of the prothoracic and mesothoracic legs, brings her wings forward until they are at a right angle to her body, and raises her metathoracic legs high over her body. If a male alights on and attempts to copulate she will thrust vigorously with her metathoracic legs and buzz her wings until the male is knocked from the copulatory position. Although the fly buzzes her wings, it is mainly the violent thrusts with her legs against the male's body which removes him. However, a persistent male may continue to make advances on a single or several unresponsive females for an extended time, 20 minutes or longer.

The first noticeable signs of this wing affliction, slight marginal tears (fig. 1-A), appear when the flies are about 3 days old. As the flies age the broken area is extended (fig. 1-B, C) until after about 3 weeks only the basal portion of the costa and possibly the alula may remain (fig. 1-D). When the wing damage becomes advanced (fig. 1-C), the insect is no longer capable of flight. These flies have been referred to as "hoppers or non-flying flies" by Starr (1953).

Observations of a dozen males after placement with gravid females demonstrated that the thrusts by the female's metathoracic legs do tear the membranous portion of the male's wings. Males, with perfect wings not previously exposed with females, after 30 minutes in a culture of gravid female flies, had numerous punctures through the membranous portion, and marginal tears at the hind edge of the wing. Once an initial break occurs in the membrane the fly will enlarge it by repeated cleaning of the broken or punctured surface. This has been demonstrated by puncturing the wing with a needle, which apparently stimulates the fly to repeatedly clean its wings, and as the legs are rubbed over the punctures, the setae on the legs snag and tear the wings more.

It is not uncommon to find as many as 50 to 75% of the male population in a culture 2 weeks old to have wings broken or torn to some degree. The percentage and degree of wing breakage increases gradually in flies 3 days to 7 days of age, after which the increase is quite sharp. It has been the belief of Barber & Starnes, that the females copulate about 3 days after emergence and again about 7 to 10 days later under laboratory conditions. Thus the data suggest that after the second copulation the females resist further advances by males. Presumably mating would occur additional times if fertilization were unsuccessful. Hutchison (1916) observed copulation taking place as late as 47 days after emergence.

The broken wing condition was first encountered in 1952 in an old breeding culture, a serious cultural defect was suspected at the time. A number of tests were conducted to determine if cultural practices would influence the percentage of wing breakage among male flies. Larval and adult food media of different nutritional value, and intervals of starvation of larvae and adults were tested with negative results. Crowding of adults in breeding cages likewise gave no solution. Ten pairs of flies confined in very small individual boxes to prevent flight, failed to mate and wing breakage did not occur. Flies in colonies of one sex only attempted copulation between individuals of the same sex, especially if the male and female cultures were side by side, which suggests that odor may stimulate sexual excitement. In these cases the passive individual, either male or female, would resist the aggressor in the same way the unresponsive females resisted the males. Since the broken wing condition occurred among colonies of either sex, it suggests that resistance against the aggressor during mating was a factor in wing breakage.

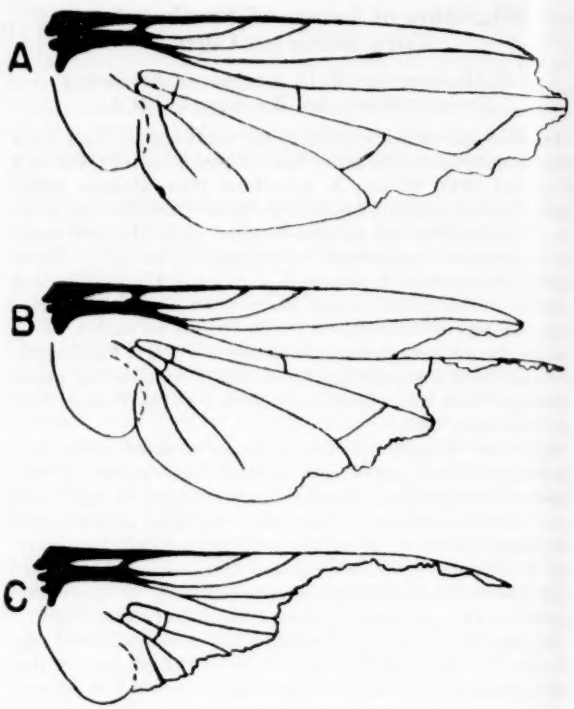

FIG. 1—Broken wings of house flies: A, after 3 days; B, after 16 days; C, after 20 days; D, after 30 days.

The broken wing condition is not confined to culture flies, as field specimens were collected which had very similar wing breakage. Since the flightless males are easy prey to the elements, few severe cases of broken wings were found in the field.

CONCLUSIONS.—The broken wing condition among flies in laboratory cultures is confined largely to males and is produced by thrusts of the flies, usually females, with the metathoracic (not mesothoracic) legs in resisting mating attempts. Females in the laboratory appear to be fertilized twice, once when about 3 days old and again when 7 to 10 days old. They resist further advances of males after the second copulation. This condition is

[1] Accepted for publication September 25, 1956.

not confined to laboratory cultures. Field specimens with similar wing breakage have been observed.

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Toxicity of DDT in Acetone and in Oil to Adult House Flies[1]

ROY J. BARKER and ABD-EL-RAHMAN RAWHY[2], *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

In studies of the toxicity of insecticides more information has been needed on the effects of solvents. Busvine (1951) found a greater difference between normal and resistant house flies in their susceptibility to DDT in acetone than in oil. To gain more information a comparison has been made on the toxicity and the absorption of topically applied DDT in acetone and in oil on the house fly, *Musca domestica* L.

The acetone used as a solvent was redistilled reagent grade. The oil was a clear, low-volatile, saturated petroleum oil (Shell Risella Oil 117).

TOXICITY.—Mortality data were obtained on 2-day-old normal adult house flies (1948 National Association of Insecticide and Disinfectant Manufacturers strain) of mixed sexes that were reared by the Chemical Specialties Manufacturers Association method at 25.5° C. and 30% relative humidity. Comparisons were made on house flies from the same populations; two populations were used. The flies were fed skim milk prior to treatment. While they were anesthetized with carbon dioxide, 1.23 microliters of the DDT solution was applied to the pronotum of each from a micrometer-driven syringe. Treated flies were held in lots of 40 in cheesecloth-covered pint jars supplied with 5% sugar water and held at 25.5° C. and 30% relative humidity. Mortality counts were made 24 hours after treatment; flies exhibiting any movement were considered alive.

To evaluate relative toxicity and to determine the precision of the estimates, the mortality data were subjected to probit analysis as described by Finney (1952). The equation showing the regression of mortality, expressed in probits, on dosage, expressed in logarithms in 0.01 microgram per fly, with oil is $Y=9.26X-11.25$ and with acetone is $Y=6.19X-1.98$.

The 24-hour median lethal dose (MLD) for DDT in oil has a maximum likelihood estimate of 0.566 microgram per fly. With a fiducial probability of 95%, its true value may be expected to lie between 0.475 and 0.675 microgram per fly. DDT-acetone solutions give a MLD of 0.134 microgram with a true value probably between 0.127 and 0.142 microgram per fly. The slope of the probit regression line for oil (9.26 ± 0.45) is steeper than that for acetone (6.19 ± 0.56).

Within an hour after treatment, the oil-treated flies had concentrated at the bottom of the holding jars but the acetone-treated flies were at the top. If little change in the ratio of DDT to oil is assumed, chemical analysis of the DDT rinsed from holding jars implies that 6.9 microliters of oil was transferred to the jar. This oil may hinder flies climbing the glass surface.

When adults of the resistant strain used in absorption studies were treated with 0.8 microgram of DDT in 1.23 microliters of acetone, 1.7% of 904 died. However, when the same amount of DDT was applied in 1.23 microliters of oil, 21.2% of 695 died. These flies are usually immune to DDT; however, the dying flies showed typical DDT tremors. When the same quantity of oil was applied with no DDT, 0.5% of 195 were killed.

ABSORPTION.—To reduce interference from mortality, a highly resistant Orlando-Beltsville strain was employed to measure DDT absorption in house flies. This strain was obtained by exposing 135 generations of the progeny of the Orlando strain to DDT. The flies were reared, treated, and held in the manner described for the NAIDM strain except that 50 flies were held in each jar. Each fly was treated with 0.8 microgram of DDT in 1.23 microliters of solvent. The dosage was verified by chemical analysis immediately after treatment. After 24 hours the flies from each jar were rinsed three times with 10 ml. of freshly redistilled ether. The rinses were combined, evaporated to dryness on a steam bath, and then nitrated for DDT analysis by the Schechter-Haller method as modified by Sternburg *et al.* (1950). The pint-jar containers were also rinsed three times with 10 ml. of ether, and the rinses of three jars were combined for DDT analysis.

Absorption of DDT in resistant flies was measured in eight replicates by subtracting the amount found in ether rinses and that rinsed from holding jars from the 0.8 microgram applied. With DDT in acetone 74.1 ± 14.6% was absorbed, and with DDT in oil 40.7 ± 11.9%. Because absorption is dependent upon dosage, these percentages are not applicable to LD-50 dosages. However, an estimate of the absorption of median lethal dosages can be based on the data of Barker (1953). The estimated absorption of MLD of 0.134 microgram in acetone is 0.131 microgram (98%), and of 0.566 microgram in oil 0.368 (65%).

CONCLUSIONS.—At the 50% mortality level at 25.5° C., topically applied DDT was 4.2 times as toxic to adult normal house flies when dissolved in acetone as in petroleum oil. The dosage-response curves were not parallel. On resistant flies, 0.8 microgram was more toxic in oil than in acetone.

More DDT was absorbed from acetone solution than from oil. The difference in absorption seems insufficient to explain the difference in mortality.

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[2] Egyptian Plant Quarantine Department. Participant, International Cooperation Administration.

Corn Earworm Lethal Factor in Silks of Sweet Corn[1]

E. V. WALTER, *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

In 1941 R. A. Blanchard of the Bureau of Entomology and Plant Quarantine and R. O. Snelling of the Bureau of Plant Industry, Soils, and Agricultural Engineering, working on resistance of corn to the corn earworm, *Heliothis zea* (Boddie), observed what appeared to be an earworm-lethal factor in the silks of an inbred line of flour corn designated as 221. Larvae placed among the fresh silks soon after hatching fed for a few hours, or even several days, and then died. Blanchard further tested this line until 1946, but was never able to find more than 14% of the infested ears that showed the lethal factor. The writer continued work with the line and made a selection that was practically immune to feeding in three successive seasons. The line was nearly self-sterile and was lost in 1950.

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Apparently the same lethal factor showed up in 1948 in another inbred, designated as 166. This inbred was selected from a cross between highly resistant 81–1 and Illinois 14n, neither of which had ever shown this character. Selections of inbred 166 have shown this character in varying degree every year since 1948.

In 1951 S. H. Yarnell of the Southeastern Regional Vegetable Breeding Laboratory at Charleston, S. C., provided several inbreds for testing for resistance to the corn earworm. His M119–8–7–4–2 was very resistant; so several ear selections from it were retained for testing in 1952. One of these selections proved to be more resistant than the others. In 1954 all of five lines descended from this selection had dead larvae in the silks. One of these lines, M119–8–7–4–2–L1–2–3, had dead larvae in 12 out of 16 ears tested. The line was not tested in 1955 owing to lack of larvae.

The lethal factor was also found in several inbred selections from a cross of a dent corn from Guatemala × teosinte × sweet corn × sweet corn. About one-third of the ears of each of two inbred selections from this cross had dead larvae in both 1954 and 1955.

In 1955, in plot tests of sweet corn for corn earworm resistance at Lafayette, Ind., 30 inbreds and several ear-row selections of some of them had dead larvae in the silks or on the tips of 5 to 50% of the ears. Twenty-six of these inbreds had the inbred 81–1 in their pedigree, and the other four had Illinois 14n. None of these inbreds and selections included the M119 selections but did include some of those derived from the teosinte cross.

Crosses have been made between several inbreds in which dead larvae have been found in the silks. All such crosses have been resistant to the earworm, and many have had dead larvae in the silks. A few crosses between these inbreds and earworm-susceptible inbreds have had dead larvae in 10 to 20% of the ears. Inbred selections from crosses having the lethal character in one or both inbred parents frequently have dead larvae. Apparently the character can be transmitted in breeding. Dead larvae found in the ears may be first instars that have fed on the silks for only a few hours or as old as third instars that fed as far down as the tip of the ear before dying. The death of larger larvae on the ear has been considered as possibly due to some disease. It is very difficult to find the dead first or second instars among the dead and brown silks of mature ears.

Occasionally larvae reach the ear and feed on the nearly mature or dry kernels after other larvae have died in the fresh silks, an indication that the lethal factor may be due to some substance that is lost or changed as the silks mature.

Corn kernels dipped in a water suspension of dead larvae removed from the silks have been fed to healthy young larvae in cages in two seasons. None of the larvae thus fed have died from a cause resembling death from the lethal factor. The fact that certain inbreds show this lethal character every year but most others have never shown it suggests that death of the larvae in the silks is not due to a virus or bacterial infection.

With the possible exception of inbred 14n, the pedigree of which is unknown, all lines that have silks showing the lethal factor trace to a southern corn ancestry. Inbred 221 was selected from flour corn from New Mexico. Inbred 81-1 was selected from a cross Honey June × Mexican June, and M119 is from a cross between a sweet corn and Cuban Yellow Flint. Those having teosinte in the ancestry also have a dent corn from Guatemala and the sweet corn inbred 81-1.

Control of *Hieroglyphus nigrorepletus* Bol.[1]

P. D. SRIVASTAVA, *Zoological Research Laboratories, University of Allahabad, India*

Hieroglyphus nigrorepletus Bol., the *juar* grasshopper, is an important major pest of *Andropogon sorghum*, *Pennisetum typhoideum* and *Saccharum officinale* and causes great losses to these crops in India. The present problem was taken up with a view to learn which stage of the grasshopper could be killed with maximum ease, by the same dosage and within the same period of time.

Different stages of the grasshopper[2] were obtained from the host plants in the field as well as from the laboratory rearings, so as to make a large number of different stages available at one time. Five per cent BHC dust (gammexane) which contains 0.65% gamma isomer, was tried against all the nymphal instars and the adult. Trials were conducted in the laboratory under as near natural conditions as possible and the results obtained gave a fair indication of the usefulness of the insecticide in the field as well. Dusting was done with Wouldron's hand duster, and leaves of *Andropogon sorghum* were given to the grasshoppers as food. Four replications of each treatment were conducted, the fifth replication in each case being the control. The initial number of insects used for each replication was 50 and the results of the observations are recorded in table 1.

Table 1.—The mortality of different stages of *Hieroglyphus nigrorepletus* treated with 5% BHC dust (Gammexane).

Stage of Grasshopper	Net Percentage of Mortality for Each Replicate after:—					
	24 hrs.		48 hrs.		72 hrs.	
	Replicates	Average	Replicates	Average	Replicates	Average
Nymphal instars						
First	70, 60, 68, 62	65	100, 92, 96, 92	95	100, 94, 100, 98	98
Untreated	0		4		4	
Second	58, 62, 56, 60	59	88, 86, 84, 92	87.5	94, 96, 98, 98	96.5
Untreated	0		0		0	
Third	40, 38, 40, 42	40	90, 92, 90, 94	91.5	94, 92, 94, 96	94
Untreated	0		0		0	
Fourth	24, 30, 34, 34	30.5	66, 70, 72, 72	70	92, 94, 90, 92	92
Untreated	0		0		0	
Fifth	16, 20, 18, 20	18.5	60, 58, 66, 62	61.5	90, 92, 90, 92	91
Untreated	0		0		0	
Adult	10, 14, 14, 14	13	40, 46, 42, 40	42	90, 90, 90, 94	91
Untreated	0		0		0	

DISCUSSION.—Different workers have tried different insecticides to control grasshoppers. Corkins (1923) used sodium arsenite whereas Granovsky (1927) and Kuznetsov (1927) applied arsenical dusts by airplane. La Rivers (1944) also used airplane dusting to control *Anabrus simplex*. Mitchener (1951) tried emulsifiable formulation of aldrin and dieldrin against *Camnula pellucida* and *Melanoplus* sp.

The author has found that 5% BHC dust successfully controls all the stages of *H. nigrorepletus*. At the end of 72 hours the average mortality of the first, second, third, fourth and fifth instar nymphs and of the adult was 98, 96.5, 94, 92, 91 and 91%, respectively. The younger stages of nymphs die earlier, for even at the end of 24 hours the mortality of the first and second instar

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[2] *Hieroglyphus nigrorepletus* (brachypterous form)

nymphs was 65 and 59% respectively, whereas that of the adults was only 13%. It is apparent therefore, that the younger stages die earlier and in larger numbers as compared with the later stages. This shows that the younger stages are less resistant to BHC. Apparently the reason for their being less resistant is that their cuticle, which is soft in younger stages, gets hardened and becomes more resistant in the later stages. The softness of the cuticle makes the penetration of the insecticide into the body of the insect easier, hence they die more easily.

SUMMARY.—Five per cent BHC dust (gammexane) caused mortality of the first, second, third, fourth and fifth instar nymphs and of the adults of *Hieroglyphus nigrorepletus* Bol. to the extent of 98, 96.5, 94, 92, 91 and 91%, respectively, at the end of 72 hours after treatment.

The mortality of the first and second instar nymphs was higher than of other stages. The nymphs of the earlier stages die earlier hence they are more susceptible to insecticides than other nymphal stages or the adults.

ACKNOWLEDGEMENTS.—The author is extremely grateful to Shri S. C. Verma, Reader in Zoology, University of Allahabad for his help and able guidance and to Dr. A. S. Srivastava, Entomologist to Government U.P., Kanpur, for his kind help and suggestions in the final preparation of the paper.

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[1] Original not seen.

The Laboratory Colonization of *Culicoides variipennis* (Coq.)[1]

ROBERT HENRY JONES,[2] *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

With the incrimination of the biting midge, *Culicoides variipennis* (Coq.), in the transmission of bluetongue of sheep in the Southwest (Price & Hardy 1954) it became desirable to colonize this species in the laboratory so that adequate experimentation could be conducted on its control and on its transmission of this disease. Wirth & Bottimer (1956) found that this species occurred in abundance in all areas of Texas where bluetongue is endemic. As it can be found in practically all *Culicoides* mud-type breeding areas in the vicinity of the Kerrville, Texas, laboratory, it is an ideal species to use in studies of these small, blood-sucking flies.

The two colonies now being maintained in the laboratory—Sonora (Edwards County), colony 3, and Klein's Pond (Real County), colony 2—are now, respectively, on their fourth and fifth reared generations. Flies are being obtained at the rate of about 100 per day from the Sonora colony.

The rearing procedure now in use is outlined in this paper. New data will probably result in some revision. The two main problems at this time are the obtaining of more viable eggs and the replacement of blood feeding on a rabbit with artificial blood feeding or with the use of an artificial diet.

The life cycle in the laboratory is about 30 days, the approximate breakdown being as follows: egg 2, larva 23, pupa 2, and preoviposition period 3. All stages are kept in a constant-temperature room at 75° F. with the relative humidity at 50%.

ADULTS.—Adult flies are retained in half-pint and gallon ice-cream cartons. The smaller size will hold up to 400 flies and the gallon size several thousand. The disk on the top of the carton is replaced with clear plastic to permit easy observation of the confined flies. Two holes in this plastic top provide for aeration and access to the cage, one covered with nylon cloth cemented on with acetone and the other stoppered with a small cork. Nourishment consists of water and sugar for the males and, in addition, blood for the females. Water is provided by saturating cotton in a small dish, and sugar by the use of split raisins. The water is contained in the bottom half of a small, round pillbox inserted snugly in a hole in the bottom of the carton.

Blood meals are furnished the females on a white rabbit, as it is a convenient laboratory animal and the flies readily engorge on the inner surface of the ears. The rabbit is confined on its back in a stock so that its ears point downward. To prevent excessive annoyance to the rabbit, the internal ear cavity is plugged with cotton. A clear plastic cylinder which barely accommodates one ear in its circumference is used to confine the flies to the inner surface. The ear is introduced through a hole in one of the pieces of white cloth that cover both ends of the cylinder. As the cylinder is about one-third longer than an ear, the flies tend to accumulate at the lower free end after leaving the ear. Cork-stoppered holes permit easy removal of the flies by means of an aspirator. The use of carbon dioxide permits the rapid transfer of large numbers to an ear.

The maximum number of flies, including both sexes, that should be placed on an ear at one time is about 500. The average time for engorgement with blood was 9 minutes (range 4 to 23) for the 62 times it was measured with individual females. An additional 10 minutes must be allowed for many of the flies to settle down and start feeding. When carbon dioxide is used, the feeding period may be as long as 1 hour, as time must be allowed for the flies to recover from being anesthetized. As most females require more than one blood meal for the production of eggs, it is necessary to blood-feed the colony every day. In tests with individuals the maximum female longevity was 37 days, this with a female that had taken nine blood meals.

Blood-feeding and mating are initiated about 1 day after the adults emerge. At blood-feeding time the males are placed in the cylinder along with the females, as the increased handling and confinement stimulate mating. Frequent matings occur in the free end of the cylinder. To increase the possibility of mating the flies are confined for 10 minutes in the 95 by 25 mm. vial of the aspirator after they are removed from the rabbit's ear, thus forcing many of them into contact by crowding. Tests with individual flies had indicated that mere confinement in the same cage was insufficient to produce mating and that forcing of the sexes into contact was necessary. The duration of mating lasted from 2 to 3 minutes.

EGGS.—The eggs are 0.4 mm. long and dark brown. Light-brown and teneral eggs never hatched, even after a month's holding. The eggs hatch about 2 days after they are deposited. Their hatching can be retarded by placement in a refrigerator, although too cold temperatures, such as actual freezing, result in decreased viability. The factor of primary importance in viability of eggs is whether or not satisfactory mating was achieved. A female is capable of depositing 675 eggs during its lifetime, the maximum number at one oviposition being 491.

The females readily deposit their eggs on the surface of the water dish. A thin piece of absorbent paper placed on this surface

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[2] The author is indebted to Willis W. Wirth and Herald M. Brundrett of this Branch for initiating this program. He is also grateful for the valuable contributions made by his former assistant, Donald E. Hopkins.

facilitates their removal to the larval pans. Oviposition usually occurs at night in the absence of light.

LARVAE.—The rearing pans are shallow, white enameled pans 7½ by 9¼ by 1¾ inches. The medium is inclined in the pan so that about one-half the surface can be maintained shallowly under water. The pans are stored in a rack permitting the automatic maintenance of the water level.

The larval medium contains 1 part of fresh cow manure, 2 parts of soil, and enough water to form a thick mud so that it can be evenly distributed in the pan. Manure from grass-fed cows is preferred, as that of grain-fed cows continues to ferment, making handling impractical. Tests utilizing sand as a base indicate that the manure in itself is sufficient for satisfactory larval development, but manure alone results in a poor condition of the medium, thus retarding larval development and increasing the difficulty of handling.

The survival, as indicated by the number of pupae recovered as compared with the number of eggs used in setting up the rearing pans, ranges up to 85%.

Under the above conditions the average length of the larval stage was 23 days, with a range of 15 to 28 days. At 85° F. and 100% relative humdity it lasted from 7 to about 12 days. However, at the higher temperature the flies are much smaller, larval mortality is higher, and it is more difficult to keep the larval medium in good condition.

PUPA.—As the pupae of *Culicoides* float to the surface of the water, they are removed from the pans by flooding with water. The larvae left in the decanted water are replaced in the pans after the excess water has been strained off with a 80-mesh sieve. The pupae are then placed in the lower half of a pillbox which is inserted into the bottom of a holding cage for emergence to the adult stage.

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Susceptibility of Different Parts of the Body of *Hieroglyphus nigrorepletus* Bol. to the Entry of Insecticides[1]

P. D. SRIVASTAVA, *Department of Zoology, University of Allahabad, India*

Hieroglyphus nigrorepletus, a serious pest of *Andropogon sorghum*, *Pennisetum typhoideum* and *Saccharum officinale*, causes great loss to these crops in India. In another paper (Srivastava 1957) the author has tried to find out at which stage the grasshopper can be killed with maximum ease. In the present paper the author has made an attempt to find out which part of the body of the grasshopper is most susceptible to the entry of insecticides.

The test insects (brachypterus form of *H. nigrorepletus*) belonged to the same developmental stage and were approximately of the same age. For this purpose the grasshoppers which had undergone their last molt on the same day were segregated. The insects belonged to both sexes and for each replication 50 insects were used.

Aldrin was available in 24% emulsifiable form and for the tests it was diluted to the percentage of 0.1, 0.2, 0.3, 0.4, and 0.5. A micrometer syringe was used to perform the experiments. All the insects were first transferred to a jar dipped in a basin containing ice and ice-cold water. By doing so the insects were benumbed, and from the benumbed lot, insects for treatment were taken out so that a representative lot, including both active and inactive insects, was drawn for the experiments. The head of the micrometer was turned through one division of the horizontal scale, when 0.06 c.c. of the liquid was passed out of the syringe needle. A measured quantity (0.06 c.c.) of the insecticide was then applied to different parts (coxa, trochanter, femur, tibia, tarsus and pretarsus) of the hind pair of legs of the grasshopper.

Experiments were performed in large cages in the laboratory at room temperatures varying from 25° C. to 35.5° C., after the treated food was given in the form of leaves of *Andropogon sorghum*. Five replications of each test were conducted; the fifth replication in each case being the control. For each replication 50 insects were taken. When 0.5% aldrin was introduced on coxa, trochanter, femur, or tibia, it caused mortality up to 30% and when introduced on the tarsus, up to 50%. Therefore, the effect of the introduction of aldrin on the pretarsus only, has been recorded in table 1.

Table 1.—Mortality of adults of *Hieroglyphus nigrorepletus* caused by different dilutions of aldrin when introduced on the pretarsus of the hind pair of legs.

	Net Percentage of Mortality after—					
	24 hrs.		48 hrs.		72 hrs.	
Dilution	Replicates	Average	Replicates	Average	Replicates	Average
0.1	4, 0, 0, 8	3	4, 4, 0, 8	4	10, 4, 2, 8	6
Untreated	0		0		0	
0.2	10, 8, 8, 6	8	22, 16, 16, 14	17	28, 16, 20, 16	20
Untreated	0		0		0	
0.3	32, 30, 34, 34	32.5	56, 58, 54, 62	57.5	60, 60, 58, 62	60
Untreated	0		0		0	
0.4	52, 56, 58, 56	55.5	86, 90, 92, 84	88	88, 90, 92, 90	90
Untreated	0		0		0	
0.5	64, 54, 70, 56	61	96, 92, 98, 96	95.5	96, 96, 100, 100	98
Untreated	0		0		0	

DISCUSSION.—During the course of the tests it was observed that the mortality did not exceed 30%, even with 0.5% aldrin when introduced on the parts of the leg other than the tarsus and the pretarsus. When introduced on the tarsus the mortality was 50% with 0.5% aldrin, but when introudced on the pretarsus, at the end of 72 hours the percentage of mortality was found to be 60, 90, and 98 with 0.3, 0.4 and 0.5% aldrin, respectively. Data given in the table show that the mortality was highest at the end of 48 and 72 hours.

Kennedy *et al.* (1948) while dealing with the relative effectiveness of DDT, BHC, and 3,5 dinitro-orthocresol, state that "the toxic effect produced on the whole insect by applying DNOC solution directly to the legs was tentatively estimated as twice that produced by application to the head and abdomen." Slifer (1950) tried to find out the vulnerable areas on the surface of the arolia. She noted the entry of methylene blue, neutral red, crystal violet and fast green through arolia of a grasshopper by dipping them into three stains. She also noted that the

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cuticle of the arolia is soft and is easily injured by abrasion, the rest of the surface of the leg being more resistant to mechanical damage and to injury by solvents. She also found that there is a waxy coating on the surface of the arolia which could be dissolved in xylol. It was also noted by her that freshly molted nymphs are less susceptible to penetration of insecticide than the older stages because they moved less; hence the chances of abrasion are comparatively less.

The present observations are in agreement with those of Slifer showing clearly that the pretarsus region is definitely more susceptible to the entry of insecticide than the other parts of the leg. The best way to detect the penetration of insecticide through the arolia would be to study sections of arolia after staining them although it is extremely difficult to detect the insecticide in sectioned tissues by stains because the amount of material picked up by the arolium would be far less than the sensitivity of analytical methods. However, so far, no stains which can stain the insecticides in sectioned materials are known, hence the present conclusion that the entry of the largest amount of insecticide takes place through the pretarsus region, has been based entirely on the basis of mortality. The percentage of mortality in the case of coxa, trochanter, femur, tibia, and tarsus also to some extent shows clearly that besides pretarsus, the entry of insecticide can take place through other parts of the leg as well, but the penetration is less and slower—a fact of great importance in the control of grasshoppers.

SUMMARY.—Based on the mortality of the insect, the pretarsus was found to be the most susceptible part of the leg of *Hieroglyphus nigrorepletus* through which the maximum penetration of insecticide takes place, when equal amounts were applied to different parts of the leg.

When aldrin was applied to the pretarsus region 0.1%, 0.2%, 0.3%, 0.4% and 0.5% of the insecticide was able to effect the mortality of the adults of *H. nigrorepletus* to the extent of 6, 20, 60, 90 and 98%, respectively, by the end of 72 hours after application.

ACKNOWLEDGMENTS.—The author is extremely grateful to S. C. Verma, Esq., Reader in Zoology, University of Allahabad, for his guidance and to Dr. A. S. Srivastava, Entomologist to Government U.P., for his valuable help and suggestions in the final preparation of the paper.

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Relation of Mite Populations to Seasonal Leaf Nitrogen Levels in Apple Orchards[1]

ELWOOD O. HAMSTEAD, *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*, and EDWIN GOULD, *West Virginia Agricultural Experiment Station, Kearneysville*

Since 1946 mite populations in the Cumberland-Shenandoah fruit area have been noticeably greater in apple orchards that have been maintained continuously in vigorous condition. Tree vigor has improved not only with the increased use of nitrogenous fertilizer but with the almost complete shift from lead arsenate to DDT in cover sprays.

Garman & Kennedy (1949) found that the rate of production of the two-spotted spider mite, *Tetranychus telarius* (L.), on potted beans and peaches was increased by soil fertilization. In a study on tomato plants under laboratory conditions Rodriguez (1951) found that, when concentrations of all the major mineral elements in a nutrient solution were doubled, two-spotted mites produced twice as many progeny. Later (1952) he found that, although increasing the nitrogen supply tended to increase the presence of certain vitamins in the foliage, evidence on the relationship of these vitamins to mite development was inconclusive. In 1953 and 1954 a study of the effect of tree vigor on the orchard mite population was undertaken at Kearneysville, W. Va.

EXPERIMENTS IN 1953.—In February 1953 randomized single-tree plots were replicated 12 times in a block of 40-year-old Stayman apple trees. These trees were in a state of low vigor with a history of mild to low mite infestations. On February 19, to provide trees having various nitrogen levels, 3 pounds of actual nitrogen in the form of 20.5% nitrogen fertilizer was hand-broadcast under 12 trees to give a high vigor, 2 pounds under 12 trees to give a medium vigor, and 1 pound under 12 trees to give a low vigor. All the trees were sprayed the same throughout the season, and no special acaricide sprays were applied. The only introduced variable was the amount of nitrogen added. Beginning on May 4 a sample of 20 leaves was collected from each tree every 14 days through the season to September 16. The mites on these leaves were brushed onto glass discs and counted. The leaves were then dried and ground for chemical analyses, each sample being handled in such manner that its chemical analysis could be identified with its corresponding mite count. Leaf analyses were made by the Lindner method (1944).

Table 1.—Number of mites per leaf in apple plots of high and low vigor, West Virginia, 1953 and 1954.

YEAR	HIGH-VIGOR PLOTS		LOW-VIGOR PLOTS	
	Eggs	Mites	Eggs	Mites
		Metatetranychus		
1953	9.0	5.5	9.3	4.9
1954	16.7	28.0	10.5	20.0
		Tetranychus		
1953	0.44	0.67	0.16	0.28
1954	0.02	0.05	0.01	0.01

The mite populations in the high- and low-vigor plots are given in table 1. Leaf samples showed that trees fertilized with 3 pounds of nitrogen had more than twice as many *Tetranychus* (two-spotted) mites as trees that had received the 1-pound application. The difference was significant, although the over-all *Tetranychus* population was comparatively small. *Metatetranychus ulmi* (Koch), (European red mites), showed no significant increase in high-nitrogen plots.

Analyses showed the leaf nitrogen to be 1.88% in plots receiving 3 pounds and 1.75% in plots that had the 1-pound application. Plot trees that had the 2-pound application retained the intermediate position with respect to mites and nitrogen.

Leaf determinations of phosphorus, potassium, calcium, and magnesium showed no clear relation to the mite populations. There was a slight tendency toward depression of the potassium level as the nitrogen level was increased, which is a common occurrence. It may be that the mite response to nitrogen increase was due to a change in mineral and/or nutrient balance induced by the addition of nitrogen. Whatever the reason for the response, it was decided through the remainder of the study to use percentages of leaf nitrogen as the criterion of correlation with mite populations.

There was definite correlation of the seasonal rise and fall of

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the available leaf nitrogen with that of the mite population. Leaf nitrogen in all plots reached a seasonal average high of 1.87% on July 14. *Metatetranychus* mites reached a seasonal population peak at the same time. *Tetranychus* mites reached a peak on July 27, at which time the leaf nitrogen was comparatively high but was declining. From July 14 to August 10 the leaf nitrogen declined slowly and mite populations decreased rapidly. By August 20 both mites and nitrogen had reached a low level.

Mites feeding on the leaves may have helped to bring about the nitrogen decline, or the nitrogen decline may have affected the decrease in number of mites. Batjer & Rodgers (1952) found that leaf nitrogen in Red Delicious apples normally begins a seasonal decline 50 to 80 days after full bloom, and this corresponded to the nitrogen drop shown in the Stayman plots.

EXPERIMENTS IN 1954.—The same experimental block was used in a repeat study during the 1954 season. Ownership of the orchard had changed, and the new owners increased the amount of nitrogen fertilizer used in the orchard area surrounding the experimental block, applying in February about 3 pounds of nitrogen per tree in the form of ammonium nitrate. They also used about twice as much DDT in the cover sprays as was used in 1953.

An attempt was made to widen the difference in leaf-nitrogen level between the high- and low-vigor plots by applying 4 pounds of nitrogen per tree on the high-vigor plots and allowing low-vigor plot trees to carry through the season without any additional fertilizer. The medium-vigor treatment was discontinued in order to release time for taking records on more trees (18) for each of the other treatments. Leaf samples for mite counts and nitrogen analyses were taken in the same manner as before.

As shown in table 1, *Tetranychus* mites were negligible during the season in all plots and in all surrounding orchard areas, but the *Metatetranychus* mites were greatly increased.

In high-vigor plots the mites became noticeably destructive to foliage about June 20. The increase of *Metatetranychus* over low-vigor plots was 29% for the active forms and 37% for the eggs. Seasonal average leaf nitrogen in high-vigor plots was 2.02%, and in low-vigor plots 1.5%. The seasonal peak of leaf nitrogen occurred near the end of June and the seasonal peak of mites about July 13.

On July 1 the grower applied Aramite at 1½ pounds per 100 gallons to control the mites. However, the population rise continued for 12 days but then dropped from 187 forms per leaf (75% active mites) to about 25 forms (equal numbers of eggs and active mites) in 14 days. It is probable that the Aramite spray prevented the July 13 peak from going higher than the 187 forms per leaf, but apparently it did not bring about the seasonal population drop that started after July 13. If the 1954 population is compared with that in 1953, when no special acaricide was used, it can readily be seen that the trends were the same each year.

DISCUSSION.—During both seasons the mite populations were substantially greater in plots showing the highest leaf nitrogen. The seasonal mite peak followed closely the seasonal leaf-nitrogen peak. Other factors being equal, high leaf nitrogen appeared to be a factor that could give an otherwise benign mite population enough boost to permit it to become destructive and difficult to control.

The amount of leaf nitrogen had no apparent effect on the time necessary to complete a generation of mites. The mite peaks in high- and low-nitrogen plots occurred simultaneously. The population increase in high-nitrogen plots was entirely a matter of more eggs laid and hatched.

The data show that a spray applied just after the seasonal population has started to drop could falsely be credited with giving excellent control, whereas an identical spray applied a few days earlier, when the population is still increasing, could give an entirely different reading even though the number of mite forms per leaf at the time of the application is essentially the same.

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On the Biology of the Corn Leaf Aphid[1]

JEAN JACQUES CARTIER,[2] *Kansas State College, Manhattan*

The history of the corn leaf aphid, *Rhopalosiphum maidis* (Fitch), dates back to the first recorded observation of Fitch (1856). At that time, the entomologists believed this insect was attacking the roots as well as the leaves of corn. Forbes (1891, 1894, 1905) showed that the differences between the corn leaf aphid and the corn root aphid (*Aphis*) *Anuraphis maidi-radicis* Forbes were of specific character. He experimented on the life cycle with several different host plants. All the aphids were killed by frost and no survivors overwintered. He never found males nor egg-depositing females. He observed that on young corn plants, the aphids did not stay long or reproduce. Davis (1909) listed some of the host plants, but did not mention the peculiarities of the life cycle.

The biology of the corn leaf aphid was last reviewed by Wildermuth & Walter (1932). With the exception of about 40 males obtained in their rearing experiments at Tempe, Arizona, between 1914 and 1924, and a few males occurring in these experiments, no sexual forms have been reported. The reproduction in nature appears to be exclusively parthenogenetic. Wintering in the latitudes south of northern Texas, the alate viviparous females are believed to migrate northward as the weather becomes favorable in the spring. In 1954 and 1955, the Canadian Insect Pest Review has reported very alarming outbreaks of the aphid on late barley in the central and eastern provinces of Canada. Those are the most northern important infestations known for the species.

SEXUAL FORMS.—The sexual forms of the corn leaf aphid are extremely rare. However, several males were observed in the greenhouse during March 25 and April 6, 1955. Most of them were found in an abandoned culture which had been purposely left aside to see how much poor conditions could affect the biology of the aphids. The culture concerned was one of the

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[2] Present address—Science Service Laboratory, St.-Jean, Quebec, Canada.

These observations are an introduction to a study on the resistance of sorghums to the corn leaf aphid, a dissertation presented in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Entomology at Kansas State College.

The author wishes to express his gratitude to Dr. R. H. Painter for directing these studies.

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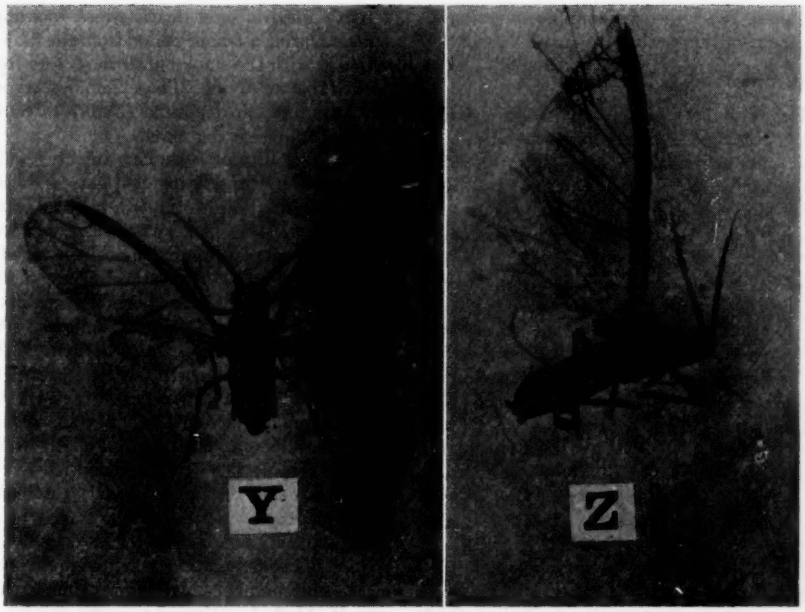

FIG. 1.—*Rhopalosiphum maidis* (Fitch), Y, dorsal view of a male weighing 0.080 mg. Z, lateral view of a male weighing 0.160 mg.

biotype KS 1 described by Cartier & Painter (1956). The biotype KS 2 has not produced males so far. Thirteen males were sent to the United States National Museum for identification. They were identified as males of *R. maidis*. Some of the specimens were kept and acknowledged as the only specimens in the Museum collection.

Figure 1 shows a dorsal view (Y) and a lateral view (Z) of the male corn leaf aphid. Figure 2 shows a sketch of the genitalia showing the penis in copulating position. The only available drawing of the male corn leaf aphid found in the literature is that by Wildermuth & Walter (1932). Their male was apparently drawn from a preserved specimen and shows some differences from specimens reared here. In their drawing the prothorax is too narrow, and the stigma of the wings appears to be set at an angle with the costa. The genital plates and the penis are not clearly outlined. The contour of the hind wing is different. Their general and taxonomic description agrees with what was observed. No other statements seem necessary regarding the number of sensoria of the antennae and the proportion of the segments of the antennae.

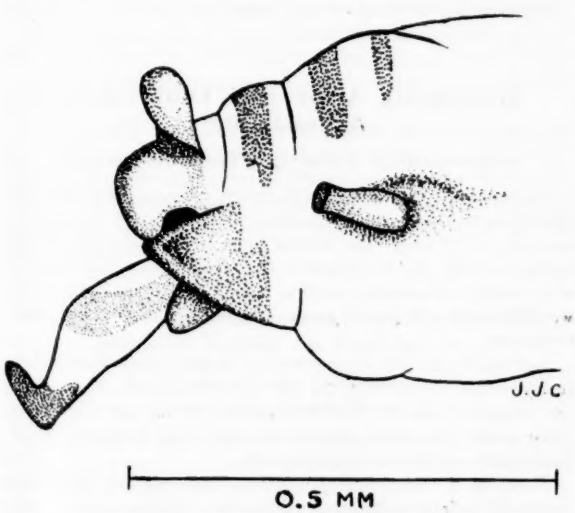

FIG. 2.—A sketch of the male genitalia of *Rhopalosiphum maidis* (Fitch), showing the penis in copulating position.

As seen in figure 1Y the dorsal view of the male shows the straight sides of the abdomen and the genitalia protruding at the tip. The cauda barely extends over the genital plates. The profile of the abdomen, as seen in figure 1Z, shows a dorsal curvature extending from the fourth to the seventh abdominal segment. This region has been seen to bend under when the males apparently exhibited copulatory reflexes.

The size of the males varied with the condition of their culture. All those obtained from the abandoned culture were very small, like the one shown in figure 1Y. The abdomen was equal in length to the thorax and the head together. A few males were obtained from another culture of the same biotype where the plant was in a normal growing condition with a small population of aphids in the cage. These males were much bigger than the other males and weighed twice as much, like the one shown in figure 1Z. This particular specimen had an abdomen which was one-fourth longer than the length of the head and thorax together. During the last instars, the nymphs of the males can be recognized by their slender and straight-sided abdomen. They already had a short prothorax, which gave them a stubby appearance similar to that of the adults. The color of their abdomen was uniform and darker than that of the other nymphs.

ACTIVITY IN RELATION TO TEMPERATURE.—In the greenhouse, at 60° F., the aphids were not seen moving; while, at 75° F., they were always active and excreting honeydew. They could withstand temperatures as high as 110° F. in the greenhouse. The optimum temperature for flight appeared to be about 74° F. since a great number were observed on the wing at that temperature during a period of cloudy weather in April, 1954. Very few flew at temperatures over 82° F. They came to lights at night, in the greenhouse, at temperatures of 70–75° F.

CARRIER OF A MITE.—Some alate females were captured at night while flying about the lights in the greenhouse. On several occasions, they were carrying as many as seven individuals of *Ereynetes* sp. (Ereynetidae) (Det. E. W. Baker), a reddish mite

abundant in the greenhouse. When the carrier aphid was caught, the mites abandoned their carrier promptly.

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Death-Stress Oviposition by *Aedes canadensis*[1]

ROBERT C. WALLIS and ROBERT J. DEBISHOP, *The Connecticut Agricultural Experiment Station, New Haven*

Death-stress oviposition by *Aedes sollicitans* (Wlk.) was described by De Coursey & Webster in 1952. They suggested that oviposition forced by insecticide application might provide enhanced control in salt-marsh areas under conditions unfavorable for egg development. They postulated that this might provide a controlling measure for other species if further research demonstrated death-stress oviposition. The purpose of this paper is to report this type of oviposition by *Aedes canadensis* (Theo.), the most common biting pest during the spring and summer season in Connecticut.

Bi-weekly collections of adult *Aedes canadensis* were obtained from Shade Swamp, Conn., during the early summer of 1956. They were divided into two experimental groups. Females containing fresh blood meals were separated from the collection and isolated in test tubes which contained water and moist filter paper strips. Cotton stoppers in the tubes were kept saturated with 5% sucrose solution. All specimens were observed daily for oviposition.

All other female mosquitoes in the collection were maintained over water in lantern chimney cages and fed sucrose solutions during a 4-week observation period. These were processed at the end of the observation period as follows: Cigarette smoke was blown into the chimneys for knock-down, and the immobilized mosquitoes were checked for identification. They were then transferred into vaccine vials which were sealed for freezing 1 hour later. The number of specimens which oviposited in the vials before freezing was recorded.

In the first group, a total number of 36 engorged females were isolated in test tubes. Of these 1 oviposited 24 eggs while in confinement, and died 8 days after oviposition. Twelve others delayed oviposition until just prior to death. Twenty-two of the females died in the tubes before eggs were developed and did not oviposit. In the second group, the 219 female *A. canadensis* maintained in lantern chimneys exhibited no sign of oviposition activity until after a death stress was applied with cigarette smoke. During a 1-hour period from the time the mosquitoes were stupified with the smoke until they were frozen in vials, 32 oviposited before dying. Those females responding with death-stress oviposition deposited from 7 to 88 eggs. There was an average of 38 eggs per female.

The possibility of enhancing insecticide control of salt-marsh mosquitoes by considering death-stress oviposition suggested by De Coursey & Webster (1952), may be extended to include the fresh-water species, *Aedes canadensis*, which exhibits death-stress oviposition similar to that described for *Aedes sollicitans*. The role of death-stress oviposition in insecticide application might be more significant than generally realized in view of recent biological studies of *Aedes* mosquitoes. Woke (1955) reported delayed oviposition by *Aedes aegypti*. Wallas & Lang (1956), by dissecting specimens of *Aedes aegypti* 14 days after a blood feeding, found that 23% retained eggs. Of these, 75% of the eggs formed were retained in a fully developed condition and could be oviposited upon application of a death-stress.

In this report 33.3% of the blood-fed *A. canadensis* isolated in test tubes delayed oviposition until just before death. However, the percentage which would be forced to oviposit in the field upon application of an insecticide would depend upon the proportion of the population which had previously obtained a blood meal. This number might approximate the percentage of the sample (14.6%) of the wild-caught specimens which were killed with smoke.

The mosquitoes utilized in this study were treated with cigarette smoke (a standard procedure for stupifying specimens for identification and preparation of mosquito pools for virus isolation studies (Aitken 1956)) rather than a standard insecticide, but they responded with death-stress oviposition just as De Coursey & Webster (1952) described for *Aedes sollicitans*, which were killed with a variety of chemical compounds.

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Forecasting Armyworm Outbreaks—A Possibility[1]

S. MARCOVITCH, *University of Tennessee, Knoxville*

The sudden appearance of armyworms (*Pseudaletia unipuncta* (Haw.) and their equally sudden disappearance arouses our curiosity as to where the worms come from, and what are the causes leading up to outbreaks. Our studies have furnished us with enough information on these questions, so that in the future we will be able to forecast armyworm outbreaks with reasonable assurance.

Due to the sporadic appearance of the armyworm there is little fundamental information on this important pest. This note is not intended to give a final forecasting system, but to point out some leads that show promise of producing more productive information on forecasting outbreaks.

There is a widespread confusion that migrant armyworm moths fly in from southern localities, in a manner similar to the fall armyworm, *Laphygma frugiperda* (J. E. Smith). The fall

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armyworm is a tropical species whose permanent breeding grounds are in southern Florida, southern Texas, and points further south. On the other hand, the true armyworm is capable of overwintering in Tennessee or anywhere in the United States or southern Canada. They generally hibernate as larvae in grassy areas.

The spring generation of moths from these larvae fly to grain fields, lay eggs in April, which upon hatching give rise to the worms that cause the usual spring outbreak. In Tennessee these outbreaks generally occur during the month of May. Thus each locality raises its own armyworms when conditions are favorable.

In Tennessee, there are five generations of worms annually, so that they are with us the year round. Under ordinary conditions, the parasites are sufficiently numerous to keep the armyworm in check. The most efficient natural enemy appears to be the small Braconid, *Apanteles militaris* (Walsh). This parasite for practical purposes is specific for the armyworm throughout the season, whereas the Tachina flies make their appearance only when the worms appear in outbreak numbers.

If for some reason, *A. militaris* is absent, an outbreak of armyworm will generally follow. Our studies indicate that dry weather rather than cold, wet springs is very detrimental to *A. militaris*. Detailed information on this point will be published later. A study of the past history of armyworm outbreaks shows that a definite pattern is followed in that they usually follow drought years. For example, the widespread drought of 1860 in the Mississippi Valley was followed by the notable and destructive outbreak of armyworms in 1861. In the same way, the extended droughts of 1895, 1913, 1933, 1952, and 1953 were all followed by destructive outbreaks in the respective following year. Even in so-called normal years of average rainfall certain localities or even individual farms will suffer from dry weather through the absence of local showers. This erratic distribution of local showers could easily explain the presence of local outbreaks that have puzzled us in the past.

A study of the temperature and moisture requirements of *A. militaris* is under way. These studies in conjunction with weather records in outbreak areas should furnish enough information to make it possible to forecast armyworm outbreaks in the future. Although Tennessee has a large annual rainfall of about 50 inches, rains are often irregular and droughts are frequent during the months of June, July, August, September, and October. Drought conditions are present when less than 0.25 of an inch of rain falls in a 21-day, or longer period. One or more such droughts during a critical period in the life of *A. militaris* may be fatal in its development, and give rise to an armyworm outbreak in the following year.

Light trap records are an additional help in forecasting. As a rule armyworms are not difficult to control provided we are forewarned while the worms are small and before real damage occurs.

It is especially desired to call to the attention of those workers who are in a position to gather data on armyworm outbreaks following drought conditions in their own localities, the possibility of forecasting such outbreaks thereby. Specimens of armyworms sent to the author for the rearing of parasites will be highly appreciated.

Eriococcus azaleae Comst. and *Cerococcus kalmiae* Ferris on Cranberry[1]

W. E. TOMLINSON, JR., *Cranberry Station, University of Massachusetts*

The azalea bark scale, *Eriococcus azaleae* Comst., was reported as a cranberry pest by Franklin in 1952. At that time it was also noted that an apparently undescribed species of *Cerococcus* was commonly associated with azalea bark scale infestations. Observations since that time have corroborated that these insects are often associated on the same bog, conditions that have favored one have evidently favored the other. Since then, however, the azalea bark scale has been of much less importance on cranberries than the *Cerococcus* scale.

FIG. 1.—*Cerococcus kalmiae* Ferris crawlers newly settled at leaf nodes. ×12.

Specimens of the *Cerococcus* scale were submitted to Dr. Harold Morrison, U.S. Department of Agriculture, Entomology Research Branch, Insect Identification Section, in March 1955, who identified them as *Cerococcus kalmiae* Ferris, a species recently described from specimens collected on mountain laurel, *Kalmia latifolia* L., at Haverford, Pa.

The azalea bark scale and *C. kalmiae* have damaged only bogs that are never flooded. Since most Massachusetts bogs are flooded at least during the winter months, damage caused by these insects has not been extensive. Both species are single brooded. *C. kalmiae* matures and lay eggs in the fall within a light brown parchment-like covering. The winter is spent in the egg stage. Eggs hatch during the latter half of May. The crawlers migrate to leaf nodes on both old and new growth (fig. 1) where the survivors settle, maturing in late summer (fig. 2). Eggs of *C. kalmiae* are gray and the young crawlers are very dark brown.

The azalea bark scale spends the winter as a partially grown scale, usually at crotches in the vines. They mature in the spring at which time the eggs are laid within the characteristic cottony covering the scale produces. Eggs of this species hatched during the latter part of June, a month or more later than those of *C. kalmiae*. Eggs and crawlers of azalea scale are purple and are easily distinguished from *C. kalmiae* when both species occur together.

Since 1952 the azalea bark scale has not caused serious damage to cranberries. However, since that time three separate bogs have required treatment to control infestations of *C. kalmiae* because of the serious leaf drop and vine killing that it caused.

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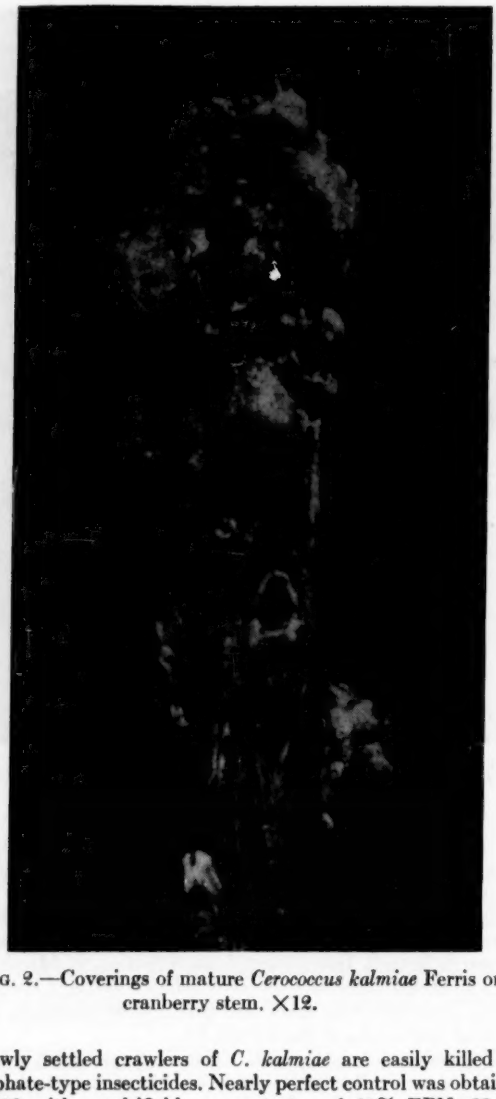

FIG. 2.—Coverings of mature *Cerococcus kalmiae* Ferris on cranberry stem. ×12.

Newly settled crawlers of *C. kalmiae* are easily killed by phosphate-type insecticides. Nearly perfect control was obtained in 1953 with emulsifiable concentrates of 45% EPN, 33.3% Metacide and 57% malathion, each at 1–800, and 15% parathion wettable powder at 2 pounds per 100 gallons of water. All sprays were applied at the rate of about 400 gallons per acre to insure complete coverage of the vines. None of these sprays injured the cranberry plants.

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Lamprophorus tenebrosus Introduced into Guam to Combat the Giant African Snail[1]

GEORGE D. PETERSON, JR.[2]

The giant African snail, *Achatina fulica* Bowdich, became es tablished on Guam during World War II, where it soon became a serious pest of several important food crops and some ornamental plants. Various methods of control were developed which included the use of molluscicides, barriers, and the predatory snail, *Gonaxis kibweziensis* E. A. Smith. This snail was introduced into Guam from Agiguan (Aguijan), Mariana Islands in September, 1954. Pemberton (1954) gives a brief history of the *Gonaxi* project on Agiguan which was conducted by the Pacific Science Board of the National Research Council.

The Indian glow-worm, *Lamprophorus tenebrosus* (Walker), has long been known to be an important enemy of the giant African snail in Ceylon and India. Studies of this interesting lampyrid made by Paiva (1919) and Hutson & Austin (1924) promoted considerable interest in the possibility of utilizing this predator in controlling the snail in other areas where it has become established.

During February and March, 1955, six air express shipments of *L. tenebrosus* larvae were received from Ceylon and liberated at the following locations on Guam: 116 at Almagosa Springs; 252 at Agana Springs; 134 in the lower Talofofo River Valley; and 431 near Ylig Bay. These larvae were collected by Dr. Henry A. Bess, University of Hawaii, who was making an intensive study of *Lamprophorus* in Ceylon.

The shipping containers consisted of light wooden boxes approximately 6 by 6 by 18 inches in size. The boxes were divided into several compartments which were loosely filled with dampened wood shavings or sphagnum moss in which the larvae were packed. Ventilation was provided by means of screened holes along two sides of the boxes. Mortality averaged about 30% for each shipment.

The glow-worms were transferred to large glass jars in the laboratory upon arrival on Guam where they were allowed to feed for several hours on juvenile *Achatina*. The glow-worms fed voraciously after their long journey. It was observed that they invariably consumed the snails entirely. This was in contrast to the findings of Hutson and Austin who report that *Lamprophorus* larvae fed mainly on liquids secreted by *Achatina* and on the semi-liquid contents of the intestine. After feeding and reconditioning, the glow-worms were liberated in the field.

Extended studies of the glow-worms were not possible because they did not survive well in the laboratory. However, some observations are of considerable interest. One large, fully-matured glow-worm was confined for 10 days on moist earth in a plaster of Paris cage. During that period it consumed eight *Achatina* which varied in length from 20 to 40 mm. The glow-worm did not attack larger snails even when starved. Living *Gonaxis* adults were also caged with this larva, but were not attacked by it.

In another interesting experiment 6 immature glow-worms were confined in a plaster of Paris cage with 6 juvenile *Achatina* and 6 juvenile *Gonaxis* of about equal size. The predatory snails devoured 2 of the *Achatina* and 1 of their own species. The other 4 *Achatina* were consumed by the glow-worms. Although the glow-worms were continuously confined with the five remaining *Gonaxis*, and no additional *Achatina* were introduced into the cage, they made no attempt to attack the predatory snails, and they eventually died of starvation. This experiment indicates that *Lamprophorus* would not be predaceous on *Gonaxis* in the field.

The release areas were surveyed several months after liberation of the glow-worms, but there was no evidence of establishment.

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[1] Accepted for publication November 5, 1956.

[2] The author was staff entomologist for the Guam Department of Agriculture during the period April, 1951 to May, 1955. He is now with the University of California Agricultural Extension Service, El Centro.

Cattle Grubs Found in the Gullets of Oregon Cattle[1]

A. R. ROTH, *Entomology Research Branch, Agr. Res. Serv., U.S.D.A.*

Much of the research on the cattle grubs *Hypoderma bovis* (L.) and *lineatum* (De Vill.) has dealt with their life history and habits outside the host. Hinricksen (1888) was one of the first workers to observe *Hypoderma* larvae in the spinal cord, and Curtice (1890) found the first larvae in the esophagus. More recently Haberman *et al.* (1949) presented data on the occurrence of *bovis* larvae in the spinal canal, and Lee & Rogoff (1954) reported the results of examinations of gullets in South Dakota for the presence of *lineatum*. It is generally conceded that the larvae spend much time in these areas before migrating to the back, but little is known about the season of their occurrence and development there. Such information should prove of value in the study of systemic insecticides and in practical control programs.

For a year beginning in September 1954 the author collected cattle gullets and examined them for the presence of *lineatum*. Usually two collections were made each month, and a total of 558 gullets were obtained. They were taken to the laboratory, where they were split lengthwise and the different layers of tissue were examined over an illuminated glass plate.

Most of the gullets were taken from young Herefords, since they are usually more heavily infested than older animals, especially of dairy breeds. The abattoir was located at Albany, Ore., and most of the cattle came from the Willamette Valley.

The number of gullets examined each month and the number and length of larvae found are presented in table 1. The data clearly show that in the Willamette Valley the larvae concentrate in the gullets during the fall, from 2 to 4 months after they enter the host. The peak of development appeared to be reached in January, although some larvae reached maximum length (16 mm.) in December. This was more or less expected, since encystment in the backs of animals usually starts around the first of January. Larvae in the gullets declined in numbers rapidly during January and February owing to migration to the back, were absent from March through June, and reappeared in small numbers in a few animals during July. In February several animals had larvae in both the back and gullet. From July through September there was a gradual increase in the number of infested animals and in the number and size of larvae in the gullets.

Table 1.—*Hypoderma lineatum* larvae found in the gullets of cattle.

Date	Number Examined	Per Cent Infested	Number of Larvae per Gullet		Length of Larvae, Millimeters	
			Range	Average	Range	Average
1954						
September	55	25.4	1–27	2.5	5.5– 8.0	6.6
October	22	59.0	2–39	4.9	5.0–11.0	8.3
December	45	35.5	3–31	3.71	7.0–16.0	12.3
1955						
January	50	30	1–14	1.1	12.0–16.0	13.6
February	42	2.3	1– 1	.3	13.0–13.0	13.0
March	56	0	0– 0	0	0 –0	0
April	40	0	0– 0	0	0 –0	0
May	38	0	0– 0	0	0 –0	0
June	62	0	0– 0	0	0 –0	0
July	48	4.2	3– 3	.08	2.5– 4.5	4.0
August	53	5.6	1–14	.32	4.0– 7.0	6.0
September	47	14.8	2–30	1.08	4.5– 9.0	6.4

Close examination of larvae in several gullets indicated no definite direction of movement. Usually most of the larvae were found at the lower end of the gullet near the rumen. Greatest tissue damage was closely allied with the greatest accumulation of larvae in the lining of the gullet. One gullet examined on September 9 was found to have no larvae under the lining, but four larvae were found on the outer surface of the esophageal muscularis.

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[1] Accepted for publication October 26, 1956.

OBITUARY

Gordon Mansir Bentley
1875-1954

Professor G. M. Bentley died in Knoxville, Tennessee, on October 8, 1954. He had retired as State Entomologist and Professor of Entomology at the University of Tennessee in 1949. A native of Massachusetts, Professor Bentley graduated from Cornell University in 1900 and received the A. M. degree in 1901. After teaching for a few years in New York and North Carolina he came to Tennessee in 1905 and contributed much to the agriculture of the state through many bulletins and articles on insect control and through his activities in various organizations of nurserymen, beekeepers, florists, quarantine officials, teachers, biologists and others. He was at one time affiliated with 33 organizations.

In all of Professor Bentley's work—whether it was entomology, ornithology, photography or something else—there was a spirit of great sincerity and enthusiasm. He was an inspirational teacher, and his students will never forget the way he led them on collecting trips or his lectures on honeybees as he opened a hive. He liked to talk about his contacts with world-renowned entomologists such as Professor Comstock and Dr. L. O. Howard.

He was a charter member of the former Entomological Society of America and was active in the affairs of the American Association of Economic Entomologists for many years, serving as a vice-president on two occasions.

Professor Bentley is survived by his wife, two daughters and five grandchildren.

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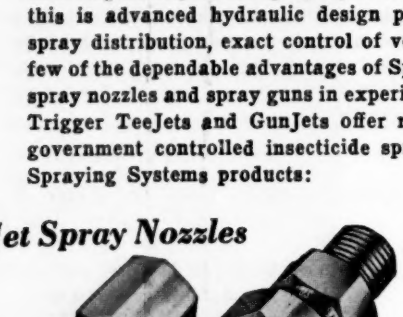

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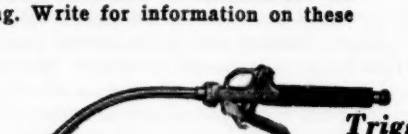

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